THE GENETICS AND CYTOLOGY OF A TETRAPLOID FROM OENOTHERA FRANCISCANA BARTLETT[1]

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Tetraploid plants of Oenothera have appeared in a number of lines under investigation and from unrelated material. The first plant, *gigas* from *Lamarckiana*, was discovered by DE VRIES (1901) in 1895 and he records that *gigas* also arose from seed of *sublinearis* in 1898 and from the cross *lata*×*hirtella* in 1899 although the latter of these two plants did not mature. MACDOUGAL (1907) reports the appearance of one plant of *gigas* from his cultures of *Lamarckiana* and SCHOUTEN (1908) lists three plants from commercial seed of *Lamarckiana*. HUNGER (1913) from large cultures of *Lamarckiana* grown in Java records five plants of *gigas*. HERIBERT-NILSSON (1909, 1912) described a plant from Swedish *Lamarckiana* with characters chiefly those of *gigas*, and GATES (1915) determined its tetraploid chromosome count. GATES (1913) identified as *Lamarckiana gigas* a line from the botanic garden of Palermo and showed it to be tetraploid. A dwarf, *gigas nanella*, from the original line of *gigas* has been studied by DE VRIES (1901, 1915), and SCHOUTEN (1908) obtained a number of these plants. BOEDIJN (1920) established a dwarf tetraploid from *Lamarckiana* mut. *simplex* of DE VRIES. Making allowance for the fact that chromosome counts were not made on some of the plants early reported as *gigas* there are still substantial records of the appearance of this tetraploid from *Lamarckiana* through a number of different lines.

Tetraploids out of species of Oenothera other than *Lamarckiana* or its derivatives have been recorded from several sources, the most important of which are the following: BARTLETT (1915a, b) reported *gigas* from *Oenothera stenomeres* and *O. pratincola*. STOMPS (1916) reported tetraploids from the cross *Lamarckiana gigas*×*atrovirens*. A line of *grandiflora* gave tetraploid plants to DE VRIES (1918) and to BOEDIJN (1924). STOMPS (1925) obtained from a cross between *biennis* and *biennis cruciata*, a *biennis gigas*, and crosses of this tetraploid with *biennis cruciata* gave *biennis cruciata gigas*. Plants of *biennis cruciata gigas sulfurea* were shown by STOMPS in the exhibition garden of the Sixth International Congress of Genetics at Ithaca in 1932. There has also been recorded the appearance of tetraploids from crosses of *gigas* and *semigigas* with certain hybrids (BOEDIJN 1924). To this list may now be added a tetraploid by way of my line of *Oenothera franciscana* B, a species with all pairing chromosomes.

[1] Cytological Studies on Oenothera V. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 351.

With respect to the origin of the tetraploid Oenotheras there have been two principal and opposing views. GATES (1909a) held that "the double number of chromosomes in *O. gigas* originated soon after fertilization by the failure of a nucleus to complete its division after the chromosomes had divided, a condition comparable to the monasters of BOVERI," and this interpretation was supported by STRASBURGER (1910). This view was soon criticized by STOMPS (1912a, b) and by LUTZ (1912) and has not been favorably received by DE VRIES (1913) or generally by other workers on Oenothera cytology and genetics who believe with STOMPS that diploid gametes are developed and function. These diploid gametes may rarely meet to produce the tetraploid; more frequently they mate with normal gametes to give the well known triploid Oenotheras out of which tetraploids may come. The origin of my tetraploid from *franciscana* was by way of a triploid thus supporting the conclusions of STOMPS.

Triploid Oenotheras derived from *Lamarckiana* were first established through chromosome counts by STOMPS (1912a) and LUTZ (1912). STOMPS wrote of them under DARWIN'S term of *hero* plants and gave them the name *semigigas* and these names are found in many papers on Oenothera. Triploids may be expected to produce a much larger proportion of diploid gametes than do diploids and the chances of an origin of tetraploids by way of triploids are correspondingly greater than the possibilities of an origin from diploids direct. Recent cytological work on haploid Oenotheras by DAVIS and KULKARNI (1930), EMERSON (1929) and STOMPS (1930) has shown the manner by which meiosis may be eliminated from the history of pollen formation in Oenothera, and the early observation of GEERTS (1909, p. 52) of an embryo sac in *Lamarckiana* with 28 chromosomes appears to have been a case of similar suppression of meiosis in the ovule. Thus the failure of a heterotypic spindle to develop or to function with the consequent reconstitution of a nucleus which then passes through the homoeotypic division would give two diploid spores. Or, the suppression of the homoeotypic division following a normal heterotypic division, but after the lengthwise division of the chromosomes, would produce a diploid spore. Both of these irregularities give the same diploid chromosome set when they occur in homozygous material, but in heterozygous material spores from suppressed homoeotypic divisions would express such chromosome peculiarities as might result from segregation in the heterotypic division.

With respect to the possibility of the origin of triploids through dispermy we have the interesting report and figure of ISHIKAWA (1918) for *Oenothera nutans*×*O. pycnocarpa*, and the observations of NEMEC (1912) on Gagea. No evidence for dispermy was reported through the careful work of GEERTS (1909) and the studies of RENNER (1914) and others on the

embryo sac of *O. Lamarckiana* and other species. Neither is there known any genetical behavior of Oenothera triploids that would suggest an origin through dispermy. Since the investigations on dispermy in animal material have established irregularities of subsequent chromosome distribution and the failure of the embryo to attain maturity, usually with its early death, we may scarcely hope that dispermy will prove to be responsible for the vigorous triploids produced by plants. The rapidly increasing reports of the production of diploid gametes in plants through the suppression of the first or second divisions of meiosis offer the only evidence on which we are at present justified in drawing conclusions. GATES (1928) has, however, expressed the view that triploids have almost certainly arisen through dispermy.

There is apparently no Oenothera material known that supports the interesting views of WINGE (1917, 1932) on the origin of constant species-hybrids as tetraploids resulting from the doubling of the chromosome sets in the F_1 individual. The tetraploids of Oenothera which have come directly from diploids have behaved genetically, as far as reported, out of line with WINGE'S hypothesis and it seems more probable that they have arisen through the union of diploid gametes. This matter will be considered in the Discussion.

The present study offers the first pedigree of a tetraploid Oenothera from a diploid by way of a triploid plant with cytological observations upon the plants directly concerned.

METHODS

Except for the F_1 generation from *franciscana*×*franciscana sulfurea nana*, grown at the JOHN INNES HORTICULTURAL INSTITUTION, all of the cultures were from seeds forced to germination over wet filter paper in Petri dishes following about 24 hours of alternate exhaust and pressure while soaking in vials of water. This treatment very much hastens germination apparently through the removal of air from the pores of the seed coats and the forcing of water against the contents of the seeds. Generally almost all of the viable seeds have germinated within 10 days and the remainder usually germinate during the next week. Seedlings are removed as they germinate to other Petri dishes and the residue in the first dish constitutes the mass of sterile seeds. When large enough to handle easily with the forceps the seedlings are planted in flats. There is little mortality among the seedlings, those that die being generally weaklings that are unable to free themselves without assistance from the seed coats. I greatly appreciate the kindness of Prof. BATESON who in 1925 gave space in the gardens at Merton for a set of cultures upon which I made observations and selfings.

It is fortunate that cytological material was collected from the triploid and tetraploid of this line so that the origin of the tetraploid is known from both the genetical and cytological side. I am indebted to Dr. ELLIOT WEIER for the material of the triploid (26.51–477) which was fixed in Strong Flemming and in Zenker's fluid, and for the material of the first tetraploid (27.36–18) fixed in Allen's modification of Bouin. The material of the second tetraploid (28.34–55), collected by Dr. KULKARNI, was fixed in a modification of Bouin somewhat stronger in chromic acid and urea and proved particularly good (Bouin's fluid 100 cc, 1 g chromic acid, 2 g urea).

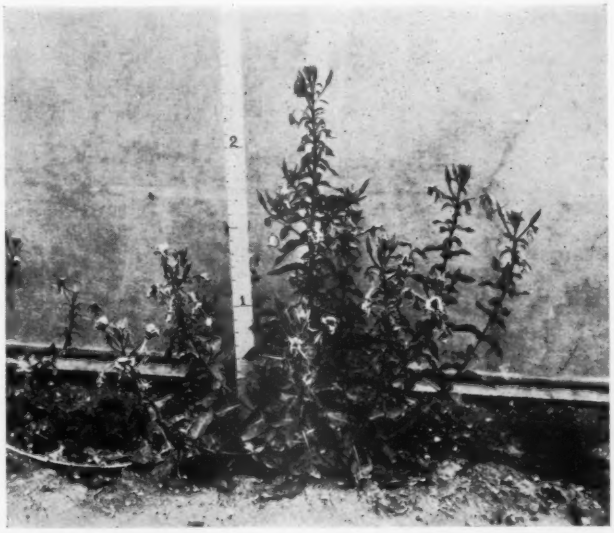
Iron alum haematoxylin gave its usual dependable results but the best preparations were stained by a modification of the Gram method as recommended by NEWTON. The procedure for this material was as follows: (1) Stain for 20 minutes in a solution of crystal violet or gentian violet, 1 g in 100 cc water. (2) Rinse in water and then for 30 seconds move the slide about in a solution of 1 g iodine and 1 g potassium iodide in 100 cc 80 percent alcohol. (3) Carry slide very rapidly through alcohols to clove oil. (4) Remove clove oil with xylol. The differentiation of the stain takes place chiefly in clove oil. If it proceeds too rapidly hasten the dehydration and for clearing use a mixture of one-third clove oil and two-thirds xylol. If destaining is too slow alternate clove oil with absolute alcohol. Chromosomes should be violet and the cytoplasm almost colorless. The chief advantage of this stain over haematoxylin lies in the translucence of the chromatic material making it possible to focus into lower levels of crowded structures.

GENETICS OF THE TRIPLOID AND TETRAPLOID

For a number of years I have been gathering data on the inheritance of three characters of *Oenothera franciscana* Bartlett in contrast with their absence in a series of derivatives from an original cross between *franciscana* and *O. biennis* L. These characters are (1) petal color whether yellow or sulfur (a cream color), (2) stature whether tall or dwarf, and (3) color of bud cones whether with red or wholly green. In the course of this work there appeared a segregate which is sulfur in flower color and dwarf in stature, in contrast to *franciscana* which has yellow flowers and is tall; both forms have bud cones with red, the gene for which is linked with that for stature. The derivative will be called *franciscana sulfurea nana* and it differs from *franciscana* in the absence of one gene that produces yellow flowers and another gene that gives tall stature. I believe that each of these characters, color and stature, depends on the presence or absence of a single gene, but certain peculiar ratios of segregation from crosses have led to experiments not yet completed in an attempt to account for marked deficiencies of sulfur and some deficiencies of dwarfs among the segregates.

The genes for color and for stature are in different chromosomes since they segregate independently from all crosses in which they are brought together.

It is necessary to make this explanation because the triploid, established by cytological study, which produced the tetraploid was one of four similar "hero" plants in an F_2 generation from the cross *franciscana*×*franciscana sulfurea nana*. These hero plants (probably all triploids) have appeared in the progeny of a number of different crosses and also once in the selfed line of *franciscana* (culture 24.21a). The triploid then is not a very rare sport.



TEXT FIGURE 1.—*Oenothera franciscana gigas*, a tetraploid arising by way of a triploid selfed, the latter from the F_2 of the cross *franciscana*×*franciscana sulfurea nana*.

Both *franciscana* and *franciscana sulfurea nana* are homozygous with sets of 14 pairing chromosomes as shown for the first plant by studies of CLELAND (1922) and KULKARNI (1928), and for the second plant by a study of EMERSON (1928), observations which have several times been confirmed in our laboratory. These chromosomes segregate freely in meiosis. The pollen of both plants is 99 percent good and the seeds are about 90 percent viable.

With respect to the characters under consideration the genetic formula for *franciscana* may be expressed as *YYTT* and for *franciscana sulfurea nana yytt; Y* stands for yellow and *T* for tall, *y* for sulfur and *t* for dwarf.

The cross with which the line started was *franciscana* × *franciscana sulfurea nana*, and the F_1 generation, *VyTt*, was grown at the JOHN INNES HORTICULTURAL INSTITUTION in the summer of 1925. This culture matured 199 plants uniform and *franciscana*-like although a little taller and with somewhat larger leaves and flowers, the latter a slightly deeper shade of yellow perhaps due to thicker petals. The pollen of these F_1 plants was 99 percent good, and the seeds 95 percent viable.

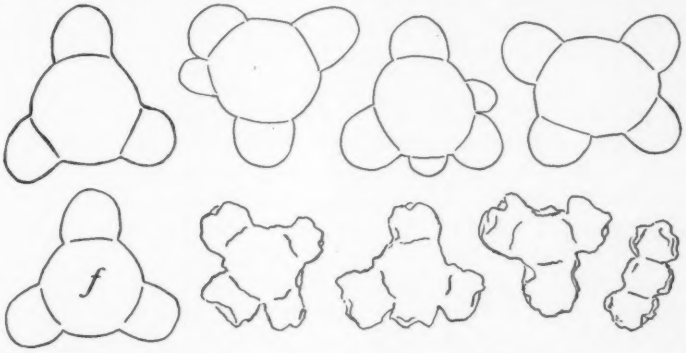
The F_2 generation from seeds of a selfed F_1 plant, (*VyTt*), germination 95.3 percent, consisted of 643 seedlings (culture 26.51, table 1) of which 617 plants were brought to maturity. These were distributed as 498 yellow tall including the 4 hero plants (triploids), 98 yellow dwarf, 16 sulfur tall and 5 sulfur dwarf. The expected segregation, assuming complete fertility of gametes and zygotes, would be 1 sulfur to 3 yellow and 1 dwarf to 3 tall; the actual ratios were 1 sulfur to 28.4 yellow and 1 dwarf to 5 tall, a very marked deficiency of sulfur and a large deficiency of dwarf segregates.

The plant (26.51–477) selected from the four hero types for selfing and which cytological study showed to be a triploid (21 chromosomes) must have arisen from the union of a normal 7 chromosome gamete with a gamete carrying 14 chromosomes. Since the triploid was yellow tall and the tetraploid derived from it was yellow tall and both types threw no segregates for sulfur or dwarf it may be assumed that the 7 chromosome gamete was *YT* and the 14 chromosome gamete *YTYT*. The *YT* gamete, which is the normal gamete of *franciscana*, should constitute one fourth of the gametes developed by the F_1 hybrid *YyTt*. The *YTYT* gamete could only have arisen through the suppression of the homoeotypic division of a *YT* nucleus segregated by the heterotypic division, the 7 chromosomes of which by their lengthwise division previous to interkinesis becoming 14 in number and the nucleus in consequence *YTYT*. Fortunately we know that such a suppression of the homoeotypic division through irregularities of spindle development may take place in Oenothera for it was observed in the haploid derived from *franciscana* (DAVIS and KULKARNI 1930, p. 73, figure 36).

The triploid plant (26.51–477) was easily distinguished in the field by its stature, taller than *franciscana*, its leaves larger, and by the much thicker bud cones. Only about 30 percent of the pollen in the triploid was well formed of which about half of the grains were 3-lobed and the other half 4-lobed with occasionally a 5-lobed example. The other grains were much shriveled and empty of material, and they also fell into two groups of 3-lobed and 4-lobed grains. Text figure 2 gives some examples of the pollen taken from this triploid plant (26.51–477) and for comparison there is included a pollen grain of *franciscana* (*f*) under the same magnification as the representatives of the triploid. It will be noted that the 4-lobed grains

are larger than those of *franciscana;* they probably carry chromosome counts above the 7 of *franciscana* but not the double set of 14. Pollen grains with 14 chromosomes from the tetraploid (2 sets of *franciscana*) as shown in text figure 3 are very much larger; they are formed very rarely and none chanced to be on the slide preserving the pollen from this triploid plant. Experience has shown that triploids in this material can be recognized with certainty by the peculiarities of the pollen as described above.

The triploid (26.51–477) selfed gave the small harvest of 513 seeds from 11 capsules, less than one-tenth the harvest of *franciscana*, and of these seeds only 116 proved viable, a germination of 22.6 percent. There was heavy mortality among the seedlings and young rosettes such that only



TEXT FIGURE 2.—Samples of pollen from the triploid plant (26.51—477), parent of the tetraploid (27.36—18). Of the 30 percent of good pollen about one-half of the grains were 3-lobed and the other half 4-lobed. A grain of *franciscana* (f) is shown for comparison. ×200, reduced one-third.

89 plants lived (culture 27.36, table 1). These were distributed as 3 tetraploids, 42 franciscana, 17 dwarfs with narrow leaves and small flowers, and 27 dwarfs of a variety of form that failed to mature.

One of the three tetraploids (27.36–18), which will be called *Oenothera franciscana gigas*, was selected as parent of a tetraploid line and material was collected for the cytological study which established the count of 28 chromosomes. It must have arisen from the union of two 14 chromosome gametes each consisting of two sets of *franciscana* chromosomes. The tetraploid is then to be interpreted as carrying four sets of *franciscana* chromosomes and with respect to the factors for flower color and stature to have the formula *YTYTYTYT*. The 42 *franciscana* plants in the same culture (27.36, table 1) resulted from the union of gametes bearing the single *franciscana* set of 7 chromosomes. These conclusions are based on the findings of the cytological study of the triploid plant (26.51–477), parent of the

culture, in which segregation from the trivalents during meiosis gave occasionally a distribution of 14 but very much more frequently a distribution of 7 chromosomes. The mixed assemblage of dwarfs probably reflected irregularities of chromosome segregation characteristic of the triploid.

The tetraploid, text figure 1, is not quite so tall as *franciscana* but the leaves are much larger and thicker, the bud cones much broader, and the flowers larger. The capsules are shorter and thicker expressing a yield of seeds per capsule less than half that of *franciscana*. In general the tetraploid may be described as more robust than *franciscana* as may be seen by comparing text figure 1 with the published figure of *franciscana* (DAVIS and KULKARNI 1930, p. 57). The pollen is about 20 percent well formed and these grains have 4 or more lobes; very rarely a 3-lobed grain is produced. As shown in text figure 3 the good grains are generally larger than those of the triploid, text figure 2, and very much larger than the pollen grain of *franciscana* (f). The remainder of the pollen consists of shriveled empty grains of various sizes, mostly 4-lobed. As in the case of the triploid, experience has shown that the tetraploid may be recognized with certainty by the peculiarities of its pollen so that an examination of the pollen will separate tetraploids from triploids when other distinguishing characters are less clearly evident.

The first tetraploid plant (27.36–18) of the line produced 159 seeds from 5 capsules which gave a progeny of 59 seedlings (culture 28.34), a germination of 35.9 percent. There matured 41 tetraploids uniform and true to the type; 7 dwarfs failed to flower. The pollen of the plant (28.34–55) selfed for the next generation proved to be 10–20 percent good.

The second generation of the tetraploid line (culture 29.47), from 349 seeds out of two capsules, started with 227 seedlings, a germination of 65.3 percent. There matured 218 tetraploids, uniform; one variant, a broad-leaved dwarf, failed to flower. The plant (29.47–113) selfed for the next generation presented pollen 20 percent good.

The third generation of the tetraploid line (culture 30.41) from 210 seeds out of one capsule, began with 146 seedlings, a germination of 69.5 percent. There matured 129 tetraploids, uniform; 6 narrow-leaved dwarfs failed to flower; a single plant, *franciscana*-like, probably came from the parthenogenetic development of an egg. The plant (30.41–90) selfed to continue the line had pollen 20 percent good.

The fourth generation of the tetraploid line (culture 31.51) from 324 seeds out of one capsule started with 92 seedlings, a germination of 28.4 percent. There matured 54 tetraploids and one plant with narrower leaves. The plant (31.51–43) selfed for the next generation had pollen 10 percent good.

TABLE 1

Pedigree of the triploid and tetraploid.

CULTURE	PARENT	PERCENT OF GERMINATION	SEEDLINGS	MATURE PLANTS	FAILED TO FLOWER	DIED	GOOD POLLEN IN SELFED PLANT	NUMBER OF SEEDS PER CAPSULE ON SELFED PLANT
F_1 generation J.I.H.I. 1925	*franciscana* × *franciscana sulfurea nana*			199 *franciscana*-like	40 *franciscana*-like		99 percent	About 500
F_2 generation (26.51)	F_1 plant grown at J.I.H.I.	95.3	643	498 Yellow tall, including 4 triploids 98 Yellow dwarf. 16 Sulfur tall 5 Sulfur dwarf 617	3	23	Triploid (26.51–477) 30 percent	Triploid (26.51–477) About 50
From the triploid (27.36)	Triploid 26.51–477	22.6	116	3 Tetraploids 42 *franciscana* 17 Dwarfs, narrow-leaved, small-flowered 62	27 Dwarfs	27	First tetraploid (27.36–18) 10 percent	First tetraploid (27.36–18) About 32
From first tetraploid (28.34)	Tetraploid 27.36–18	35.9	59	41 Tetraploids	7 Narrow-leaved dwarfs	11	Second tetraploid (28.34–55) 10–20 percent	Second tetraploid (28.34–55) About 175
From second tetraploid (29.47)	Tetraploid 28.34–55	65.3	227	218 Tetraploids	1 Broad-leaved dwarf	8	Third tetraploid (29.47–113) 20 percent	Third tetraploid (29.47–113) 20 percent
From third tetraploid (30.41)	Tetraploid (29.47–113)	69.5	146	129 Tetraploids	1 *franciscana*-like 6 Narrow-leaved dwarfs	10	Fourth tetraploid (30.41–90) 20 percent	Fourth tetraploid (30.41–90) About 300
From fourth tetraploid (31.51)	Tetraploid 30.41–90	28.4	92	54 Tetraploids 1 Narrower-leaved 55		37	Fifth tetraploid (31.51–43) 10 percent	Fifth tetraploid (31.51–43) About 125

The chief points in this account of the history of the triploid and tetraploid are summarized in table 1. An interesting feature is the marked uniformity of the flowering populations from the tetraploids throughout the four generations. The few backward plants and dwarfs probably resulted from some unusual chromosome distributions, and the high percentages of shriveled pollen undoubtedly expressed in the main the irregularities of meiosis that break up the *franciscana* sets. The relatively small harvests of seed produced mean similar high percentages of sterility among the ovules. The true breeding of this tetraploid with respect to the plants that mature stands in marked contrast to that of *gigas* from *Lamarckiana* which always gives, when completely represented, a highly varied progeny. This contrast in behavior probably rests on the heterozygous constitution of the *gigas* derived from *Lamarckiana* in contrast to the more stable situation in the tetraploid from the homozygous *franciscana*.

CYTOLOGY OF THE TRIPLOID

There was not sufficient material of the triploid (26.51–477) to make profitable a study of the earlier prophases of meiosis in the microsporocyte. This account will then begin with the period of the second contraction which, as shown in plate 1, figure 1, presents a thick chromatic thread for the most part closely gathered but with some loops or arms extending outward from the central mass. The appearance suggests that synaptic associations have already taken place as becomes evident when the loops and other portions of the spirem separate as the nucleus passes out of second contraction. Segments of the chromatic thread are then clearly shown as chromosomes, in pairs and in groups of three (figures 2 and 3). Chains of three segments which are to become trivalents are numerous (figure 3), but no nucleus was observed in which the 21 chromosomes were in 7 chains of 3 each. Nevertheless, such must occur since 7 trisomes were found on a few spindles at metaphase of the heterotypic division (figure 5). Generally there is a mixture of pairs, groups of three, and solitary segments.

Further condensation of the segments gives the short chromosomes characteristic of the heterotypic division and these are assembled in association with a multipolar spindle (figure 4). The chromosomes are mostly in trivalents and bivalents which become very clear as the equatorial plate is established. This is as one would expect in a triploid with all pairing chromosomes. Occasionally a spindle carries 7 trivalents as shown in figure 5 where three of the trisomes from a lower focus are drawn at one side. Usually both bivalents and trivalents are present, as in figure 6, which gives 5 trisomes and 3 pairs; the members of one of the pairs must be a chance association of odd chromosomes that failed to mate with their homologues. Sometimes one or more chromosomes lie quite apart appar-

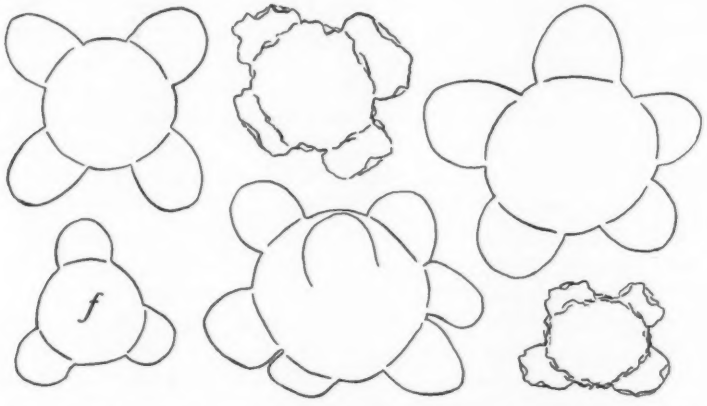
ently having failed to establish associations with others. The three chromosomes of a trivalent may be grouped in one of two ways as shown in figure 5; either (1) there may be a third chromosome attached to one member of a pair as $\frac{ab}{ba}$ or (2) in a chain of three the middle chromosome is bent so that the two end chromosomes lie parallel although not in conjugation as *ab ab*. Either way it is evident that two chromosomes will pass to one pole and the third to the other pole. When 7 trisomes are present they rarely lie so that the segregation is 7 and 14 although such segregation must take place since nuclei were found at interkinesis with 7 split chromosomes (figure 8), and at the end of the homoeotypic mitosis with 14 chromosomes (figure 16). Usually the trivalents lie with some of the single chromosomes pointing to one pole and some to the other as illustrated in figure 5 where segregation would have given two groups of 9 and 12 chromosomes respectively. Assuming no irregularities of trisome formation and no lagging of chromosomes the mathematical probabilities of a segregation of 7 and 14 chromosomes from a set of 7 trisomes would be 1:128. Thus a very large percentage of the segregation is sure to be irregular even when a full set of trisomes is present.

Other examples of irregularities of segregation are shown in figures 7 and 9 where the distribution of the chromosomes is 10–11 and 8–13 respectively, and figures 10, 11, 12 and 13 give nuclei at interkinesis with counts of 9, 10, 11 and 12 chromosomes. Some of this irregularity results from the fact that very many heterotypic spindles present fewer trisomes than the maximum of 7, the other chromosomes being in pairs (figure 6), or loosely associated. On such spindles the chances of the segregation of sets of 7 and 14 chromosomes is much less than when the full number of trisomes is present.

Interkinesis (figures 8–13) presents the split chromosomes characteristic of Oenothera in preparation for the homoeotypic division. During prophase of this division the chromosomes become further condensed (figure 14), and at metaphase (figure 15), the daughter chromosomes are usually distributed in equal sets. Consequently a tetrad of microspores will generally have two nuclei with one chromosome number and two of another as determined by the heterotypic division, the sum of the two numbers being 21. Thus from the nucleus of figure 14 would have come two nuclei of a tetrad each with 9 chromosomes, and from the division of figure 15 two nuclei each with 12 chromosomes. Rarely are nuclei in tetrads found with 14 chromosomes (figure 16), but the union of gamete nuclei of this character produces the tetraploid.

Another factor leading to irregular distribution of chromosomes is the

lagging and consequent separation of occasional chromosomes from their proper groups. When this takes place on the heterotypic spindle there may be found during interkinesis 4 nuclei in a cell, two large and two small (figure 18), the latter containing the chromosomes that failed to reach the poles. Such small nuclei following the heterotypic division were not found to pass into the homoeotypic division. Similar small nuclei may be formed from chromosomes that lag during the homoeotypic division (figure 17). The final results of such failure of the normal distribution of chromosomes are sporocytes usually with 6 nuclei (figure 19), of which two are much smaller than the others. This gives the not uncommon condition of polyspores such as have been reported for other triploids and for various hybrid material.



TEXT FIGURE 3.—Samples of pollen from the tetraploid plant (27.36—18), parent of the tetraploid line. The 20 percent of good pollen showed grains much larger than those of *franciscana* (f), and with 4 or more lobes. ×200, reduced one-third.

The behavior of the chromosomes during meiosis in the triploid makes clear the breeding peculiarities of this plant. As shown by the records of culture 27.36 (table 1), the progeny from the triploid plant was 3 tetraploids, 42 franciscana, 17 dwarfs with narrow leaves and small flowers, and 27 dwarfs that failed to flower. The large and strong plants were then products of the union of 14 chromosome gametes to give tetraploids and the union of 7 chromosome gametes to give *franciscana*. We do not know the chromosome organization in the dwarfs but they probably came by way of gametes with chromosome counts between 7 and 14. The 70 percent of shriveled pollen presented by the triploid would seem to be amply covered by the irregularities of chromosome distribution during meiosis, the smallest shriveled grains being from cells with nuclei of low chromosome

count. Nuclei with large chromosome numbers might also produce shriveled grains if the chromosome assortments were of an unfavorable character.

In connection with the large number of dwarfs there must also be taken into consideration the high mortality of plants in the field (culture 27.36, table 1) and the low seed germination of 22.6 percent. These data indicate that many gametic combinations fail to give viable zygotes or produce weaklings. The large number of *franciscana* plants thrown by the triploid shows that the segregation of the 7 chromosomes constituting the *franciscana* set must take place with much greater frequency than the segregation of two sets of *franciscana* chromosomes necessary for the gametes that may produce the tetraploid. This is to be expected since the accidents of irregular distribution are more likely to lessen the frequency of the diploid sets of 14 chromosomes than of the haploid sets.

CYTOLOGY OF THE TETRAPLOID

The cytology of the tetraploid has been studied from two plants (27.36–18 and 28.34–55) of the first and second generation. Stages from the first plant are given in figures 20–29 and from the second plant in figures 30–34. The account of meiosis will make use of both sets of figures for there was no difference in the behavior of the two plants. The equatorial plate of a somatic mitosis from a developing petal with its 28 chromosomes is shown in figure 20.

The chromosomes emerge from second contraction in short chains as illustrated in figure 21 where about 22 chromosomes are evident, the remaining 6 probably being hidden in the central chromatic mass. The chromosomes then become more evenly distributed in a stage corresponding to diakinesis, but they are still in short chains or occasional pairs as shown in figure 22 where 23 chromosomes are now quite free and only 5 remain in the central group. No examples were found of complete pairing during diakinesis which must take place rarely if at all. An early spindle of the heterotypic division is presented in figure 23 where the chromosomes appear some in pairs and some in short chains. There seemed to be no marked tendency to form tetravalents although the tetraploid must contain 4 sets of homologous chromosomes.

Metaphase of the heterotypic division is shown in figure 24 with most of the chromosomes regularly paired but with trivalents and unpaired chromosomes also present. Figure 30 from the second tetraploid is particularly interesting because all of the 28 chromosomes could be drawn from different levels; there appear to be 7 pairs, a chain of 5, a chain of 4, a chain of 3, and 2 chromosomes probably not associated. The appearance of groups of 4 and 5 chromosomes probably means that short chains have

not yet broken apart as pairs of chromosomes. The large number of chromosomes apparently makes it difficult for all to find synaptic mates and consequently odd chromosomes are frequently present. These conditions lead to irregularities of meiosis and must be responsible for much of the bad pollen found in the tetraploids.

No heterotypic metaphases were observed with all of the chromosomes paired but such pairing and subsequent disjunction or at least a regular segregation must take place since counts of 14 chromosomes were found in nuclei at interkinesis (figure 25). An early telophase of the heterotypic division is shown in figure 31 with 14 chromosomes in each daughter nucleus. Prophases of the homoeotypic division with 14 split chromosomes are illustrated in figures 26 and 27, and in figure 32 a metaphase is shown with 14 split chromosomes on each of the two spindles which lay at right angles to one another. A telophase of the homoeotypic division with 14 chromosomes is given in figure 33. These examples show conditions that would lead to the production of the very small amount of pollen with 14 chromosomes capable of reproducing the tetraploid line.

There is much distribution of chromosomes by the heterotypic division in counts above and below the number 14 but judging from the highly true breeding of the tetraploid such chromosome groups apparently rarely produce fertile pollen. The failure of chromosomes to find their synaptic mates is responsible as in the triploid for a large amount of lagging on the heterotypic spindles and this results in irregularities of segregation. Lagging also occurs in the homoeotypic divisions (figure 28), similar to that which has been described for the triploid, and small nuclei may be developed (figure 29), with the consequent formation of polyspores. There seems to be enough of these irregularities to account for the large proportions of shriveled pollen grains.

It is rather surprising that the tetraploid should present a smaller percentage of good pollen than the triploid. Perhaps the explanation lies in the larger number of chromosomes concerned which make it less easy for a chromosome to meet its appropriate mate. The second, third and fourth tetraploids of the line (table 1) apparently had better pollen than the first and also produced more seeds per capsule, but the fifth tetraploid was about as highly sterile as the first so there is no evidence of improved fertility in the line.

There is shown in figure 34 a sporocyte which apparently contains a restitution nucleus following the suppression of the heterotypic division, in which case the nucleus should contain 28 chromosomes. The chromatin is much distributed and there is no indication of the approach of the homoeotypic division. The example, however, suggests that irregularity characteristic of the haploid from *franciscana* (DAVIS and KULKARNI

1930) by which two pollen grains may be formed in a sporocyte following the suppression of the heterotypic division.

The stability of the tetraploid line is remarkable. The few variants that appeared (table 1) were almost all dwarfs that failed to flower. The parent *franciscana* so regularly thrown by the triploid has not been represented in the cultures of the tetraploid which means that it is difficult if not impossible to segregate a set of the 7 *franciscana* chromosomes from the 28 chromosomes or four sets in the tetraploid. The homologous chromosomes of the tetraploid endeavor to associate in pairs and there appears to be no opportunity for a single set of 7 to detach itself from so large a group. This will, however, happen in the triploid whenever a full set of trisomes are so placed on the heterotypic spindle that the third chromosomes of the groups all face one pole.

Variations of chromosome segregation in the tetraploid present themselves in numbers greater or less than 14 rather than between 7 and 14 as in the triploid. Such irregularities of chromosome distribution, expressed through these higher numbers, might be expected to result in greater disturbances of cell physiology with the consequent production of a higher proportion of shriveled pollen in the tetraploid than in the triploid, and this is markedly the case.

DISCUSSION

We have noted that the first triploids observed to arise directly from an Oenothera species were those from *Lamarckiana* established by chromosome counts of STOMPS (1912a) and LUTZ (1912). LUTZ also obtained triploids from *lata* and from *lata*×*Lamarckiana*, and STOMPS (1912b) reported a triploid from the cross *biennis cruciata*×*biennis*. These were important observations since they led to a series of studies which have established the fact that species of Oenothera not infrequently produce triploid sports in contrast to an early contrary opinion of GATES (1913). Later studies of which those on Morus, Datura, Hyacinthus, Tulipa, Solanum, Zea, Crepis, and Petunia are examples have shown that triploids are not rare in various groups of plants and support the conclusions on their importance as a stage in the development of higher polyploids.

The first study of the distribution at meiosis of the 21 chromosomes of a triploid Oenothera was that of GATES (1909b) on the hybrid *lata*×*gigas*. He described a segregation of chromosomes during pollen formation usually of 10 and 11, but occasionally of 9 and 12. In a later paper on the cross *rubricalyx*×*gigas* GATES (1923) again records the usual segregation as 10 and 11 with, however, irregularities that result from the lagging of chromosomes. In none of these studies on triploids did GATES describe or clearly figure the pairing of chromosomes at the time of the heterotypic division.

In this respect a paper of GEERTS (1911) on the cross *lata*×*gigas* stands in contrast to the observations of GATES. GEERTS reported a pairing between two set of 7 chromosomes with 7 chromosomes unpaired both in vegetative cells and at the heterotypic division. The behavior, therefore, was described by GEERTS as of the Drosera type, but unfortunately the study was not developed in detail. VAN OVEREM (1922) for *semigigas* also reported as usual a grouping of chromosomes in 7 pairs with the remaining 7 in a scattered distribution. There is general agreement of these investigators and also of later workers with the conclusions of GATES that the more common segregation of chromosomes is that of 10 and 11, but genetical studies indicate that the small percentages of fertile gametes produced are chiefly in a group of lower chromosome numbers, 7 or 8, or in the diploid number of 14 chromosomes.

A triploid from *Oenothera pycnocarpa* stated by CATCHESIDE (1930) to segregate its chromosomes, usually 10 and 11 or more rarely 9 and 12, was also described as presenting at diakinesis a closed chain of 21 chromosomes. Such an arrangement, if true, would be fundamentally antagonistic to the theory of segmental interchange between non-homologous chromosomes as applied to Oenothera cytology and genetics. This account of a closed chain of 21 chromosomes was promptly challenged by DARLINGTON (1930b) and by CAPINPIN (1930), and DARLINGTON after an examination of CATCHESIDE's slides interpreted them as showing various associations of chromosomes in pairs, in trisomes, and in other arrangements including single elements. CATCHESIDE (1931), accepting the interpretations of DARLINGTON, has abandoned his position.

The first descriptions of trivalent chromosomes in Oenothera material were those of HAKANSSON (1926, 1930, 1931) for the triploid plant *Lamarckiana excelsa* and for certain trisomic derivatives from *Lamarckiana*. CAPINPIN (1930) is to report in detail on a number of triploids from *Lamarckiana* and other species such as *franciscana*, *ammophila*, *biennis* et cetera, none of the triploids being derived from crosses involving either triploid or tetraploid forms. CATCHESIDE (1931) in the revision of his studies on the triploid from *O. pycnocarpa* gives good figures of chromosome associations, many of them trivalents, and an excellent discussion of their significance as possible products from a structural hybrid. The present study describes trivalents as frequent in a triploid sport from the homozygous *franciscana*, a species with all pairing chromosomes.

Thus Oenothera in presenting trivalents at meiosis in triploids agrees with the studies on Canna (BELLING 1921), Datura (BELLING and BLAKESLEE 1922), Hyacinthus (BELLING 1925a, DARLINGTON 1929b), Hemerocallis (BELLING 1925b), Campanula (GAIRDNER 1926), Solanum (LESLEY 1926, JÖRGENSEN 1928), Zea (RANDOLPH and MCCLINTOCK 1926, Mc-

CLINTOCK 1929), Prunus (OKABE 1928, DARLINGTON 1928), Tulipa (NEWTON and DARLINGTON 1929), Narcissus (NAGAO 1929), Crepis (NAVASHIN 1929), Lycoris (INARIYAMA 1931), Petunia (DERMEN 1931, STEERE 1932). These accounts of meiosis in triploids report no conspicuous regularity in the association of the chromosomes to form trivalents. There are few figures that show complete sets of trivalents such as are presented by BELLING for Canna and Hyacinthus, by MCCLINTOCK for Zea, by STEERE for Petunia, and illustrated for Oenothera in figure 5 of the present paper. The accounts generally describe and the figures present trivalents together with bivalents and univalents. Naturally the results of meiosis are most varied with the chromosomes segregated through a wide range of numbers. The lagging of chromosomes is common so that the full number frequently is not distributed to the two daughter nuclei following the heterotypic division and the lagging chromosomes may organize independent micronuclei. In consequence of these irregularities of chromosome distribution very large amounts of sterile pollen are formed.

With respect to the associations of chromosomes at meiosis in tetraploids they are bound to differ with the make up of the material whether auto- or allotetraploid. Allotetraploids with sets of dissimilar chromosomes are not likely to form quadrivalents at meiosis but autotetraploids with their four sets of homologous chromosomes may be expected to show tetrasomes. Tetravalents have been described and figured for Datura (BELLING and BLAKESLEE 1924), Aucuba (MEURMAN 1929), Solanum (JÖRGENSEN 1928, LESLEY and LESLEY 1930, LINDSTROM and KOOS 1931), Prunus (DARLINGTON 1928), Hyacinthus (DARLINGTON 1929b), Pyrus (DARLINGTON and MOFFETT 1930), Dahlia (LAWRENCE 1929, 1931), *Primula sinensis* (DARLINGTON 1931), *Rosa relicta* (ERLANSON 1931). The proportions of tetravalents present are widely various. Datura may present complete sets but in most material bivalents are found together with the quadrivalents which may be few in number.

The tetraploid from *Oenothera franciscana* failed to show more than occasional groupings of chromosomes in fours and these were in open chains at diakinesis (figures 22 and 23) and on the heterotypic spindle (figure 30). Such open chains readily break up into pairs which is the usual association of chromosomes in the metaphase of the heterotypic division. The failure of this autotetraploid to form marked tetrasomes and the predominance of pairs does not support the views on the importance of tetravalents, but rather strengthens the reports on other tetraploids where much irregularity of chromosome association is described. In this tetraploid Oenothera it is very common to find chromosomes which, having failed to meet with a mate, either associate with a pair to form a trisome (figure 24) or lie apart. The large numbers of chromosomes involved make probable a high per-

centage of such failures in synapsis with consequent irregularities of segregation.

Tetraploids from ring-forming species of Oenothera, which are structural hybrids, present conditions out of which disomes, tetrasomes, or chains might appear. Assuming *gigas* out of *Lamarckiana* (with its circle of 12 and one pair) to have the formula

ab cd ef gh ij kl mn
ab cd ef gh ij kl mn
bc de fg hi jk la mn
bc de fg hi jk la mn

the simplest arrangement of the chromosomes in synapsis would seem to be pairs such as ab ab, bc bc, cd cd, et cetera since both ends of each member of the pairs have homologues; the 4 chromosomes mn might be either as two pairs or as a tetrasome. However, chains might arise as for example cb ba ab bc which could be open or closed, short or long, possibly even up to the full number of the 24 chromosomes with interchanged segments. The only figures of *gigas* published are those of GATES (1911) and DAVIS (1911) and these indicate short chains and pairs during diakinesis and at metaphase of the heterotypic division. A re-examination of my slides supports these conclusions and the fact that *gigas* selfed gives a complex and diverse progeny indicates that the variants resulted from the numerous possibilities of irregular chromosome distribution.

In this account of the origin of a tetraploid from *Oenothera franciscana* by way of a triploid it is clear that the event must have taken place through the union of diploid gametes. There are only a few other accounts of the origin of tetraploid Oenotheras from hero plants, presumably triploids. DE VRIES (1923) gives the first record of a *gigas* individual by way of a plant of *semigigas* from a cross between *O. Lamarckiana* mut. *simplex* and *O. biennis* (Chicago). BOEDIJN (1924) reports *gigas* from *semigigas* plants out of the cross *lata* × *Lamarckiana*.

In contrast to the above are a number of accounts of tetraploid Oenotheras derived directly from diploid plants. The original *gigas* from *Lamarckiana* apparently had such an origin in the cultures of DE VRIES and other reports of *gigas* from *Lamarckiana* and from certain of its mutants give no suggestion of intermediary triploid plants before the appearance of the tetraploid. BARTLETT (1915a, b) obtained tetraploids directly from *O. stenomeres* and *O. pratincola*. DE VRIES (1918) recorded a tetraploid plant out of a line of *O. grandiflora*, also reporting *semigigas* from the same line, and *gigas* appeared in certain crosses; BOEDIJN (1924) added to this study. A tetraploid from *O. simplex nanella* of DE VRIES was established by BOEDIJN (1920). Other tetraploids have been reported by BOEDIJN as arising directly from crosses between hybrids and *gigas*. STOMPS (1925) ob-

tained a *biennis gigas* from a cross between *biennis* and *biennis cruciata*, and a *biennis cruciata gigas* from crosses of this tetraploid with the diploid *biennis cruciata*.

In view of the records of tetraploid Oenotheras out of diploid parentage it is not surprising that the hypothesis has been held by GATES and others of an origin after fertilization by a doubling of the chromosome number. This hypothesis of chromosome doubling is now best known through the extended discussion of WINGE (1917, 1932) who holds that a lack of harmony between the two sets of chromosomes in the F_1 of a wide cross might be compensated for by an "indirect chromosome binding" of daughter chromosomes following a division somewhere in the germ path, possibly in the zygote. We can conceive such a doubling to be brought about (1) by the failure of a mitosis to separate its daughter chromosomes with the immediate organization of a restitution nucleus, (2) by the fusion of two daughter nuclei, or (3) by the union of two spindles of sister nuclei lying side by side to form one spindle (WINKLER 1916) as assumed by the "endo-duplication" hypothesis of JÖRGENSEN (1928). These possibilities have their chief interest in relation to the occasional appearance of stable tetraploids, which are in effect new species, in the F_1 generations from crosses generally wide in character.

WINGE (1932) has assembled a number of cases of such stable tetraploids which apparently have arisen through the doubling of chromosome sets in the germ path of the hybrid. Good examples of this behavior are the cases of the tetraploid *Primula Kewensis* (NEWTON and PELLEW 1929), *Rosa Wilsoni* (BLACKBURN and HARRISON 1924), *Nicotiana digluta* (CLAUSEN and GOODSPEED 1925), a tetraploid from the cross *Fragaria bracteata* × *F. Helleri* (ICHIJIMA 1926), *Saxifraga Potternensis* (MARSDEN-JONES and TURRILL 1930), *Brassica napocampestris* (FRANDSEN and WINGE 1932). Outstanding features of these "constant hybrids" are their fertility and the absence of segregation which result from the harmonious relations established between the sets of chromosomes now doubled.

Support for WINGE'S hypothesis of the origin of constant hybrids through the doubling of chromosome sets in the germ path is offered in the remarkable experiments on the decapitation of stems in Solanum. Tetraploid shoots together with diploid arise from meristematic tissue in association with the callus over the wound (WINKLER 1916, JÖRGENSEN 1928, SANSOME 1930, LINDSTROM and KOOS 1931, and LINDSTROM 1932). LINDSTROM and KOOS present evidence of nuclear fusions in binucleate cells in the callus of decapitated haploids that may produce diploid shoots. An extensive literature reports the formation of nuclei with higher chromosome numbers in root tips and other tissues following experimental interference with mitosis through effects of chemicals, by wounding and by high

temperatures. Binucleate cells have been described and figured in a wide range of plant material. Tetraploid branches have appeared rather frequently in the extensive cultures of *Datura Stramonim* (BLAKESLEE and BELLING 1924). SCHWEMMLE (1928) has reported fertile tetraploid shoots on an F_1 plant of *Oenothera Berteriana*×*O. odorata*.

There are, however, serious objections to the acceptance of WINGE's hypothesis of "indirect chromosome binding" or somatic doubling as an explanation of the origin of those tetraploid Oenotheras that have arisen directly from diploids. By such origin the tetraploids should breed true and may be expected to be highly fertile since their nuclei carry two sets of homologous chromosomes brought together by the suppression of mitoses or through the fusion of sister nuclei either at rest or during simultaneous mitoses. Now such information as we have on the tetraploid Oenotheras out of diploids indicate that many of them were far from that condition of genetic stability to be expected on the hypothesis of the duplication of the chromosome sets. The *gigas* from *Lamarckiana* is well known to breed very far from true, and widely varied progenies were obtained from the Swedish *gigantea* of HERIBERT-NILSSON (1912) and the *gigas* from Palermo studied by GATES (1915). The *gigas* out of *stenomeres* (BARTLETT 1915a) produced 54 *gigas* and 9 variants in a culture of 102 plants; the remaining plants died or failed to flower indicating a high proportion of atypical forms. The *gigas* from *pratincola* (BARTLETT 1915b) gave 16 dwarfs in a culture of 25; the other 9 plants died. The *gigas* from *grandiflora* (DE VRIES 1918, BOEDIJN 1924) repeated the performance of the line in giving the forms *lorea* and *ochracea*. The *biennis gigas* (STOMPS 1925) threw dwarfs.

Thus we are forced to consider the probabilities of an origin of these tetraploid Oenotheras out of diploids through the union of diploid gametes which might result from the suppression of either the first or the second of the divisions of meiosis. The suppression of the first division permits of no segregation and the two diploid pollen grains formed give gametes which should carry the exact chromosome constitution of the diploid parent. Union of such gametes would give doubled sets of chromosomes and should a regular pairing result the tetraploid might breed true and the results would not be different from somatic doubling.

The suppression of the second division of meiosis also gives two diploid pollen grains but these will be genetically different when derived from heterozygous stock since the first division will have effected a segregation of chromosomes. There is less information on the suppression of the homoeotypic division than on the heterotypic which has been reported for material in many groups of plants and is not an uncommon phenomenon. Evidence of the suppression of the homoeotypic division is given for Pru-

nus (DARLINGTON 1930a), in hybrids of Papaver (YASUI 1931, figure 26), for Triticum hybrids (THOMPSON 1931), and for Oenothera (DAVIS and KULKARNI 1930, figures 36 and 37). HAKANSSON (1929) reports the fusion of homoeotypic spindles in *Salix viminalis*×*S. caprea*. There is probably much more of this suppression of the second division of meiosis than this scant record would suggest; studies have not been particularly directed to it.

The species of Oenothera which have given tetraploids out of diploids are probably all chain-forming types in some degree and therefore structural hybrids. Tetraploids derived from them by way of diploid gametes formed through the suppression of the second division of meiosis could hardly fail to give indications through their breeding behavior of heterozygous conditions in the parent diploids. While the union of diploid gametes formed by the suppression of the first division of meiosis may be expected to give doubled sets of chromosomes we must grant the possibility of some variation among the gametes through rearrangements of chromosome segments in the prophases of meiosis and the consequent possibility of varied progeny. The evidence in the breeding records that have come to us indicates heterozygous conditions in the tetraploids under consideration. This is the argument for the conclusion that these tetraploid Oenotheras out of diploids have arisen through the union of diploid gametes and not by a process of somatic doubling.

SUMMARY

1. An autotetraploid of *Oenothera franciscana* Bartlett has arisen by way of a triploid in the F_2 of the cross *franciscana* (*YTYT*)×*franciscana sulfurea nana* (*yytt*).

2. The F_1 of this cross was uniform, all plants (*YyTt*) were *franciscana*-like, yellow dominant over sulfur, tall over dwarf.

3. The F_2 generation (table 1) gave the expected segregation of yellow tall, yellow dwarf, sulfur tall and sulfur dwarf through combinations of the gametes *YT*, *Yt*, *yT*, *yt*. Among the yellow talls were 4 "hero" plants (probably all triploids), one of which (26.51–477) was established to be a triploid, 21 chromosomes.

4. Since the tetraploid has failed to segregate either sulfur or dwarf this triploid must have arisen from the union of a 7 chromosome *franciscana* gamete (*YT*) with a 14 chromosome gamete (*YTYT*), the latter resulting from the suppression of the homoeotypic division of a *YT* nucleus and consequently including two sets of *franciscana* chromosomes.

5. The triploid (*YTYTYT*) selfed gave a progeny (table 1) of 42 *franciscana*, 44 various dwarfs, and 3 thick-leaved yellow tall plants (probably all tetraploids), one of which (27.36–18) was established to be tetrap-

loid, 28 chromosomes. The tetraploid must have come through the union of two 14 chromosome gametes and consequently carried four sets of *franciscana* chromosomes. The tetraploid has bred remarkably true through four generations (table 1).

6. The pollen of *franciscana* is 99 percent good; the capsules set about 500 seeds, 90 percent viable. The pollen of *franciscana sulfurea nana* is equally good, capsules set about 250 seeds, equally viable. The pollen of the F_1 plants was also 99 percent good, the capsules set about 500 seeds, and the germination was equally high.

7. The pollen of the triploid was about 30 percent good and about equally divided between 3- and 4-lobed grains (text figure 2); the triploid produced about 50 seeds to the capsule of which 22 percent proved to be viable.

8. The pollen of the tetraploid is about 20 percent well formed; the 4- or more lobed grains are larger than those of the triploid and very much larger than those of *franciscana* (text figure 3); the capsules set about 200 seeds moderately viable.

9. The 21 chromosomes of the triploid emerge from second contraction in pairs and in trisomes with occasional free chromosomes. If 7 trisomes are present they may lie so that 7 chromosomes pass to one pole and 14 to the other. Such segregation is not common but it must occur since nuclei were found in interkinesis and in the homoeotypic division with 7 and 14 chromosomes respectively (figures 8 and 16). Gametes with 7 chromosomes should on union give *franciscana* in agreement with the genetical findings. Union of 14 chromosome gametes should produce the tetraploid.

10. Most trisomes lie on the heterotypic spindle of the triploid with the third chromosome pointing sometimes to one pole and sometimes to the other giving various numerical distributions of the chromosomes (figure 5). There are also the pairs and odd chromosomes to effect irregularities of segregation together with lagging chromosomes that fail to reach the poles. Interkinesis in the triploid presents the split chromosomes characteristic of Oenothera but the lagging of chromosomes in the homoeotypic division frequently disturbs further the process of meiosis.

11. These irregularities of meiosis in the triploid would seem sufficient to account for the pollen sterility of about 70 percent and for the low seed germination of about 20 percent together with large numbers of dwarfs and the high mortality among the plants in the field since it is evident that many gametic combinations fail to give viable zygotes or produce weaklings.

12. The 28 chromosomes of the tetraploid are found mostly paired at metaphase of the heterotypic division but trisomes and odd chromosomes may be present and tetrasomes are occasionally represented by short

chains (figure 30). A regular segregation of the chromosomes does take place to a limited degree since counts of 14 chromosomes were found at various stages following the heterotypic division and from such nuclei must come the small percentage of fertile pollen.

13. There is much distribution of chromosomes through meiosis in the tetraploid in counts below the number 14 as a result of lagging and through failure of many chromosomes to find a synaptic mate. These irregularities are responsible for the large amount of bad pollen in the tetraploid which is greater than in the triploid, possibly because the larger number of chromosomes present makes it less easy for them appropriately to pair.

14. A sporocyte of the tetraploid was found (figure 34) which apparently contained a restitution nucleus following the suppression of the heterotypic division.

LITERATURE CITED

BARTLETT, H. H., 1915a The mutations of *Oenothera stenomeres*. Amer. J. Bot. **2**: 100–109.

1915b Mass mutations in *Oenothera pratincola*. Bot. Gaz. **40**: 425–456.

BELLING, J., 1921 The behavior of homologous chromosomes in a triploid Canna. Proc. Nat. Acad. Sci. **7**: 197–201.

1925a Homologous and similar chromosomes in diploid and triploid hyacinths. Genetics **10**: 59–71.

1925b Chromosomes of Canna and of Hemerocallis. J. Hered. **16**: 465–466.

BELLING, J. and BLAKESLEE, A. F., 1922 The assortment of chromosomes in triploid Daturas. Amer. Nat. **56**: 339–346.

1924 The distribution of chromosomes in tetraploid Daturas. Amer. Nat. **58**: 60–70.

BLACKBURN, K. B. and HARRISON, J. W. H., 1924 The origin of a fertile hexaploid form in the *pimpinellifoliae-villosae* crosses. Brit. J. Exp. Biol. **1**: 557–570.

BLAKESLEE, A. F. and BELLING, J., 1924 Chromosomal mutations in the Jimson weed, *Datura Stramonium*. J. Hered. **15**: 195–206.

BOEDIJN, K., 1920 Die Chromosomen von *Oenothera Lamarckiana* mut. *simplex*. Z.I.A.V. **24**: 71–76.

1924 Die Gigas und Deuterogigasformen der Oenotheren. Biol. Zbl. **44**: 127–137.

CAPINPIN, J. M., 1930 Meiotic behavior of the triploid Oenotheras. Amer. Nat. **64**: 566–570.

CATCHESIDE, D. G., 1930 Chromosome linkage and syndesis in Oenothera. Trans. Roy. Soc. Edinb. **56**: 467–484.

1931 Meiosis in a triploid Oenothera. J. Genet. **24**: 145–163.

CLAUSEN, R. E. and GOODSPEED, T. H., 1925 A tetraploid glutinosa-tabacum hybrid, an experimental verification of Winge's hypothesis. Genetics **10**: 278–284.

CLELAND, R. E., 1922 The reduction divisions in the pollen mother cells of *Oenothera franciscana*. Amer. J. Bot. **9**: 391–413.

DARLINGTON, C. D., 1928 Studies on Prunus, I and II. J. Genet. **19**: 213–256.

1929 Meiosis in polyploids. II Aneuploid hyacinths. J. Genet. **21**: 17–56.

1930a Studies on Prunus III. J. Genet. **22**: 65–93.

1930b Telosynapsis or structural hybridity in Oenothera? Nature **125**: 743–744.

1931 Meiosis in diploid and tetraploid *Primula sinensis*. J. Genet. **24**: 65–96.

DARLINGTON, C. D. and MOFFETT, A. A., 1930 Primary and secondary chromosome balance in Pyrus. J. Genet. **22**: 129–151.

DAVIS, B. M., 1911 A comparison of the reduction divisions of *Oenothera Lamarckiana* and *O. gigas*. Ann. Bot. **25**: 941–974.

DAVIS, B. M. and KULKARNI, C. G., 1930 The cytology and genetics of a haploid sport from *Oenothera franciscana*. Genetics **15**: 55–80.

DERMEN, H., 1930 Polyploidy in Petunia. Amer. J. Bot. **18:** 250–261.

DE VRIES, H., 1901 Die Mutationstheorie. Leipzig.

1913 Gruppenweise Artbildung. Berlin.

1915 *Oenothera gigas nanella*, a Mendelian Mutant. Bot. Gaz. **60:** 337–345.

1918 Mass mutations and twin hybrids of *Oenothera grandiflora* Ait. Bot. Gaz. **65:** 377–422.

1923 Ueber die Mutabilität von *Oenothera Lamarckiana* mut. *simplex*. Z.I.A.V. **31:** 313–351.

EMERSON, S. H., 1928 Chromosome configuration in a dwarf segregate from *Oenothera franciscana sulfurea*. Papers Michigan Acad. S.A.L. **9:** 117–120.

1929 The reduction division in a haploid Oenothera. Cellule **39:** 159–165.

ERLANSON, E. W., 1931 Chromosome organization in Rosa. Cytologia **2:** 256–282.

FRANDSEN, H. N. and WINGE, Ö., 1932 *Brassica napocampestris*, a new constant amphidiploid species-hybrid. Hereditas **16:** 212–218.

GAIRDNER, A. E., 1926 *Campanula persicifolia* and its tetraploid form "Telham Beauty." J. Genet. **16:** 341–351.

GATES, R. R., 1909a The structure and chromosomes of *Oenothera gigas*, de Vries. Arch. Zellforsch. **3:** 525–552.

1909b The behavior of the chromosomes in *Oenothera lata*×*Oe. gigas*. Bot. Caz. **47:** 179–199.

1911 Pollen formation in *Oenothera gigas*. Ann. Bot. **25:** 909–940.

1913 Tetraploid mutants and chromosome mechanisms. Biol. Zbl. **33:** 92–99, 114–150.

1915 The mutation factor in evolution with particular reference to Oenothera. London.

1923 The chromosomes of a triploid Oenothera hybrid. Ann. Bot. **37:** 565–569.

1928 The cytology of Oenothera. Bibliogr. Genet. **4:** 401–492.

GEERTS, J. M., 1909 Beiträge zur Kenntniss der Cytologie and der partiellen Sterilität von *Oenothera Lamarckiana*. Rec. Trav. bot. néerland. **5:** 93–206.

1911 Cytologische Untersuchungen einiger Bastarde von *Oenothera gigas*. Ber. deuts. Bot. Ges. **39:** 160–166.

HAKANSSON, A., 1926 Ueber das Verhalten der Chromosomen bei der heterotypischen Teilung schwedischer *Oenothera Lamarckiana* und einiger ihren Mutanten und Bastarde. Hereditas **8:** 255–304.

1929 Die Chromosomen in der Kreuzung *Salix viminalis*×*caprea* von Heribert-Nilsson. Hereditas **13:** 1–52.

1930 Zur Cytologie trisomischer Mutanten aus *Oenothera Lamarckiana*. Hereditas **14:** 1–32.

1931 Beobachtungen über die Chromosomenbindungen bei einer triploiden Oenothera. Bot. Notiser. 1931: 339–342.

HERIBERT-NILSSON, N., 1909 *Oenothera gigas* framgangen som mutation i Sverige. Bot. Notiser 1909: 97–99.

1912 Die Variabilität der *Oenothera Lamarckiana* und das Problem der Mutation. Z. I. A. V. **8:** 89–231.

HUNGER, F. W. T., 1913 Recherches experimentales sur la mutation chez *Oenothera Lamarckiana* exécutées sous les tropiques. Ann. Jar. Bot. Buitenz. 2 ser. **12:** 92–113.

ICHIJIMA, K., 1926 Cytological and genetical studies on Fragaria. Genetics **11:** 590–604.

INARIYAMA, S., 1931 Cytological studies in the genus Lycoris. Bot. Mag. Tokyo **45:** 11–26.

ISHIKAWA, M., 1918 Studies on the embryo sac and fertilization in Oenothera. Ann. Bot. **32:** 279–317.

JÖRGENSEN, C. A., 1928 The experimental formation of heteroploid plants in the genus Solanum. J. Genet. **19:** 133–211.

KULKARNI, C. K., 1928 Meiosis in *Oenothera franciscana*. Michigan Acad. S. A. L. **9:** 223–225.

LAWRENCE, W. J. C., 1929 The genetics and cytology of Dahlia species. J. Genet., **21:** 125–159.

1931 The genetics and cytology of *Dahlia variabilis*. J. Genet. **21:** 257–306.

LESLEY, M., 1926 Maturation in diploid and triploid tomatoes. Genetics **11:** 267–279.

LESLEY, M. M. and LESLEY, J. W., 1930 The mode of origin and chromosome behavior in pollen mother cells of a tetraploid seedling tomato. J. Genet. **22:** 419–425.

LINDSTROM, E. W., 1932 A fertile tetraploid tomato. J. Hered. **23:** 115–121.

LINDSTROM, E. W. and KOOS, K., 1931 Cyto-genetic investigations of a haploid tomato and its diploid and tetraploid progeny. Amer. J. Bot. **18:** 398–410.

LUTZ, A. M., 1912 Triploid mutants in Oenothera. Biol. Zbl. **32:** 385–435.

MCCLINTOCK, B., 1929 A cytological and genetical study of triploid maize. Genetics **14:** 180–222.

MACDOUGAL, D. T., VAIL, A. M. and SHULL, G. H., 1907 Mutations, variations and relationships of the Oenotheras. Pub. Carnegie Instn. Washington **81:** pp. 92.

MARSDEN-JONES, E. M. and TURRILL, W. B., 1930 The history of a tetraploid saxifrage. J. Genet. **23:** 83–92.

MEURMAN, O., 1929 Association and types of chromosomes in *Aucuba Japonica*. Hereditas **12:** 179–209.

NAGEO, S., 1929 Karyological studies of the Narcissus plant. I. Somatic chromosome numbers of some garden varieties and some meiotic phases of a triploid variety. Mem. Col. Sci. Kyoto Imp. Univ. B **4:** 175–198.

NAVASHIN, M., 1929 Cytological investigations on triploidy in Crepis. Pub. Univ. California Agric. Sci. **2:** 377–400.

NEMEC, B., 1912 Ueber die Befruchtung bei Gagea. Bull. Inter. Acad. Sci. Bohême **17:** 1–17.

NEWTON, W. C. F. and DARLINGTON, C. D., 1929 Meiosis in polyploids. I. Triploid and pentaploid tulips. J. Genet. **21:** 1–15.

NEWTON, W. C. F. and PELLEW, C., 1929 *Primula Kewensis* and its derivatives. J. Genet. **20:** 405–467.

OKABE, S., 1928 Zur Cytologie der Gattung Prunus. Sci. Rep. Tohoku Imp. Univ. Biol. IV. **3:** 733–743.

RANDOLPH, L. and MCCLINTOCK, B., 1926 Polyploidy in *Zea Mays*. Amer. Nat. 60: 99–102.

RENNER, O., 1914 Befruchtung und Embryobildung bei *Oenothera Lamarckiana* und einigen verwandten Arten. Flora **107:** 115–150.

SANSOME, F. W., 1930 Graft hybrids and induction of polyploids in Solanum. Rep. Proc. Ninth Int. Hortic. Congress, London. **A:** 92–99.

SCHOUTEN, A. R., 1908 Mutabiliteit en Variabiliteit. Dissertation Gröningen pp. 196.

SCHWEMMLE, J., 1928 Genetische und zytologische Untersuchungen an Eu-Oenothera. Jahrb. wiss. Bot. **67:** 849–876.

STEERE, W. C., 1932 Chromosome behavior in triploid Petunia hybrids. Amer. J. Bot. **19:** 340–357.

STOMPS, T. J., 1912a Die Entstehung von *Oenothera gigas* de Vries. Ber. deuts. bot. Ges. **30:** 406–416.

1912b Mutation bei *Oenothera biennis* L. Biol. Zbl. **32:** 521–535.

1916 Ueber den Zusammenhang zwischen Statur und Chromosomenzahl bei den Oenotheren. Biol. Zbl. **36:** 129–160.

1925 Sur *Oenothera biennis* mut. *gigas* une nouvelle mutation tetraploide. Cellule **36:** 233–254.

1930 Ueber parthenogenetische Oenotheren. Ber. deuts. bot. Ges. **48:** 119–126.

THOMPSON, W. P., 1931 Cytology and genetics of crosses between fourteen and seven chromosome species of wheat. Genetics **16:** 309–324.

VAN OVEREEM, C., 1922 Ueber Formen mit abweichender Chromosomenzahl bei Oenothera. Beih. bot. Zbl. **39:** 1–80.

WINGE, Ö., 1917 The Chromosomes. Their numbers and general importance. C. R. Lab. Carlsberg. **13:** 131–275.

1932 On the origin of constant species-hybrids. Svensk Bot. Tidskr. **26:** 107–122.

WINKLER, H., 1916 Ueber die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Z. Bot. **8:** 417–531.

YASUI, K., 1931 Cytological studies on artificially raised interspecific hybrids of Papaver III. Unusual cases of cytokinesis in pollen mother cells in an F_1 plant. Cytologia **2:** 402–419.

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 (num. aper. 1.30) in combination with the compensating oculars K20 or K10. In reproduction the figures are reduced one-third from the magnification of 1600 diameters except for figures 18, 19 and 34 where the reduction is from 800 diameters.

PLATE 1

FIGURE 1.—Triploid. Second contraction, synaptic associations indicated.

FIGURE 2.—Triploid. Chromosomes shortly after second contraction, a short chain of four is shown together with pairs.

FIGURE 3.—Triploid. The period corresponding to diakinesis. Trivalents are present together with bivalents.

FIGURE 4.—Triploid. Multipolar spindle of the heterotypic division showing trivalents and bivalents.

FIGURE 5.—Triploid. Metaphase of the heterotypic division with 7 trivalents. As arranged 12 chromosomes would pass to one pole and 9 chromosomes to the other.

FIGURE 6.—Triploid. Metaphase of the heterotypic division, 5 trivalents and 3 bivalents.

FIGURE 7.—Triploid. Anaphase of the heterotypic division, 11 chromosomes at one pole and 10 at the other.

FIGURE 8.—Triploid. Interkinesis, 7 split chromosomes.

FIGURE 9.—Triploid. Interkinesis, 8 split chromosomes in one nucleus and 13 in the other.

FIGURE 10.—Triploid. Interkinesis, 9 split chromosomes.

FIGURE 11.—Triploid. Interkinesis, 10 split chromosomes.

FIGURE 12.—Triploid. Interkinesis, 11 split chromosomes.

FIGURE 13.—Triploid. Interkinesis, 12 split chromosomes.

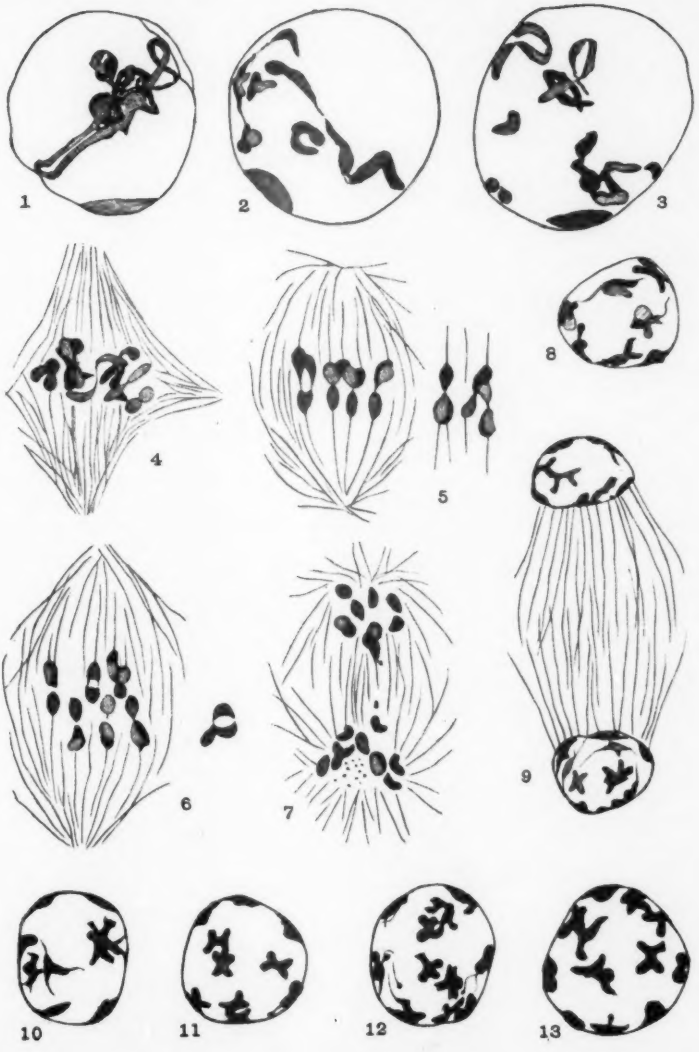

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PLATE 2

FIGURE 14.—Triploid. Early spindle of homoeotypic division, 9 split chromosomes.

FIGURE 15.—Triploid. Metaphase of homoeotypic division, 12 split chromosomes.

FIGURE 16.—Triploid. Telophase of homoeotypic division, 14 chromosomes. The union of two gamete nuclei of this character would produce the tetraploid.

FIGURE 17.—Triploid. Heterotypic anaphase with lagging chromosomes which would probably have organized two micronuclei.

FIGURE 18.—Triploid. Interkinesis with two meganuclei and two micronuclei, the latter derived from lagging chromosomes such as are shown in figure 17.

FIGURE 19.—Triploid. Polyspores, the result of lagging chromosomes that form micronuclei.

FIGURE 20.—First tetraploid. Somatic mitosis from developing petal, 28 chromosomes.

FIGURE 21.—First tetraploid. Chromosomes emerging from second contraction in short chains, about 22 evident.

FIGURE 22.—First tetraploid. Early diakinesis, chromosomes in short chains, 23 clearly defined, probably 5 in the central group.

FIGURE 23.—First tetraploid. Early spindle heterotypic division, chromosomes in pairs and in short chains.

FIGURE 24.—First tetraploid. Metaphase of heterotypic division, chromosomes mostly bivalents, some trivalents and univalents.

FIGURE 25.—First tetraploid. Interkinesis, 14 split chromosomes.

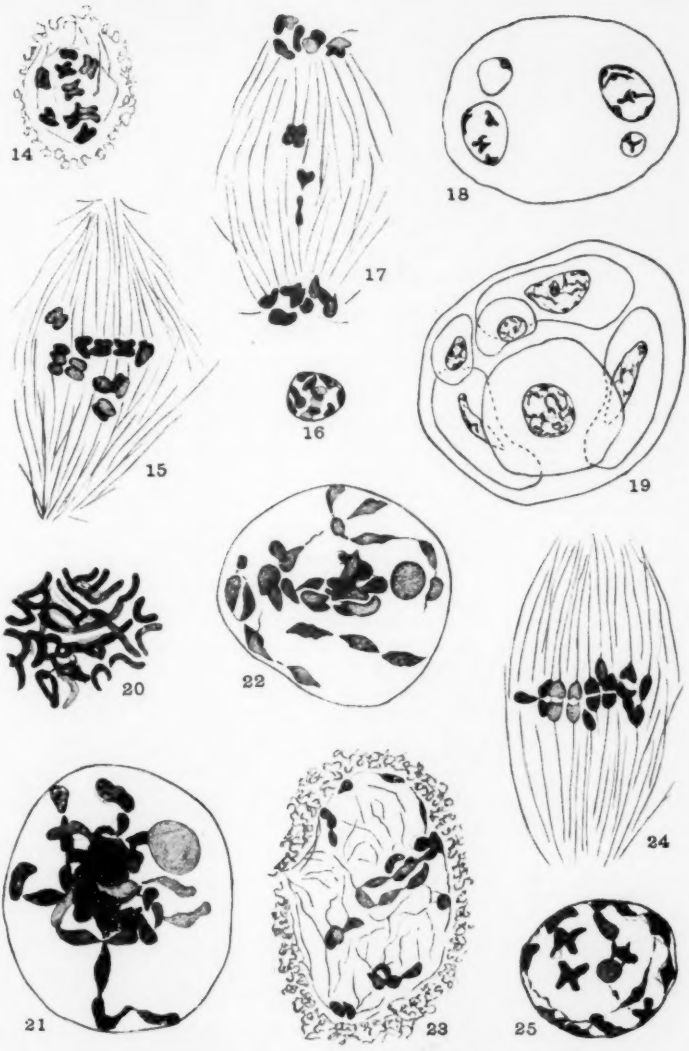
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PLATE 3

FIGURE 26.—First tetraploid. Prophase homoeotypic division, 14 split chromosomes.

FIGURE 27.—First tetraploid. Multipolar spindle homoeotypic division, 14 split chromosomes.

FIGURE 28.—First tetraploid. Homoeotypic telophase, lagging chromosomes between the daughter nuclei.

FIGURE 29.—First tetraploid. Mega- and micronuclei following the homoeotypic division, the former with 12 chromosomes, the latter probably formed from 2 lagging chromosomes.

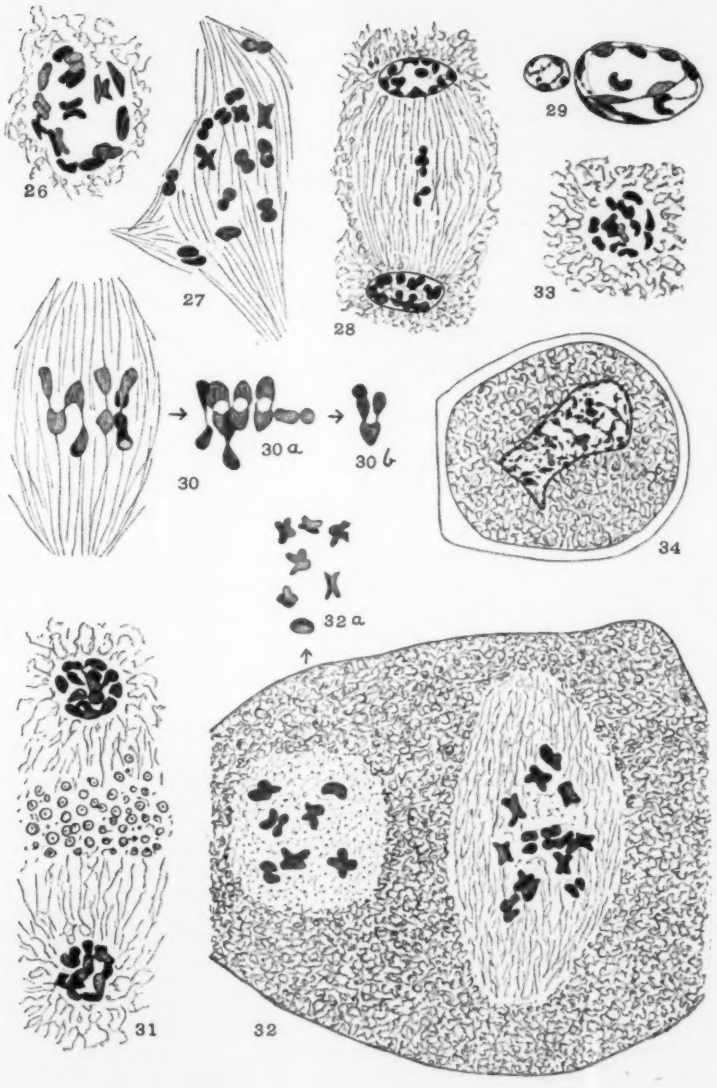
FIGURE 30.—Second tetraploid. Metaphase heterotypic division, the 28 chromosomes drawn at different foci; there appear to be 7 pairs, a chain of 5, a chain of 4, a chain of 3, and 2 chromosomes probably not associated.

FIGURE 31.—Second tetraploid. Early telophase heterotypic division, 14 chromosomes in each daughter nucleus, a zone of starch grains between the daughter nuclei.

FIGURE 32.—Second tetraploid. Early homoeotypic division the two spindles at right angles to one another, 14 split chromosomes on each spindle. Such a sporocyte should produce a tetrad of fertile pollen grains of the tetraploid genotype.

FIGURE 33.—Second tetraploid. Early homoeotypic anaphase, 14 chromosomes. This should become the nucleus of a fertile pollen grain of the tetraploid genotype.

FIGURE 34.—Second tetraploid. Apparently a sporocyte in which the heterotypic division has been suppressed giving a very large restitution nucleus.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GENETIC OBSERVATIONS ON THE GENUS LINARIA

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A few years ago, I obtained seeds from eighteen presumably different species of the genus Linaria—chiefly through the kindness of Professor Doctor E. BAUR and of HAAGE und SCHMIDT—in order to determine the value[1] of this group for genetical investigation. The list of species follows, together with some notes on their compatibility with each other.

1. *L. bipartita* Willd. Hab. northern Africa. Erect, branching, annual type. Fls. large, violet-purple, with orange palates above, becoming whitish toward the base. Spurs long and curved. Closely related to Nos. 7, 9, 10, 14, and probably will cross with them and give fertile hybrids. No crosses were obtained when the plants were used as female with Nos. 3 (12 pol.) and 17 (16 pol.).

2. *L. canadensis* Dumont. Hab. New Brunswick, New England, and south to southwest. Slender, erect, annual. Lvs. linear. Fls. small, violet-blue to purple. Late flowering. No crosses tried because of this point.

3. *L. Cymbalaria* Mill. (Kenilworth ivy). Hab. Europe. Four types grown, received under the names *vulgare* (trailing), *alba* (trailing with white flowers), *globosa* (bushy), and *compacta* (bushy). A trailing, glabrous plant, with reniform-orbicular, 5–9 lobed leaves. Fls. small, axillary, of various shades of purple above and of yellow at the lip. Spurs short. No crosses

[1] Earlier, I have made similar surveys of other genera; but, as no especially interesting contributions to genetic knowledge resulted, the results were not published. I now believe that this decision was a mistake. Short papers by geneticists who have had experience with various genera, setting forth the advantages and disadvantages of the material in question, might result in saving the time of other workers. I give two examples from my own experience.

A collection of 28 species of Begonia was made, and nearly 1000 interspecific crosses were attempted. Numerous cytological examinations were also made. Cytologically, the genus is poor material, the species having between 20 and 60 small chromosomes. In general, 1 hybrid was obtained for each 100 pollinations. No hybrids were obtained between the fibrous-rooted and the tuberous-rooted types. Hybrids between species belonging to different sections of the genus were not obtained. Even within the various sections hybridization was extremely difficult. Nevertheless, the plants have a distinct genetic advantage in ease of asexual reproduction; and, as is shown by the success of horticultural work, a considerable number of hybrids can be obtained between species that are closely related (presumably having the same chromosome number). A complete cytological survey of the genus would be very helpful to the horticulturist and possibly to the geneticist, though as genetic material the group is poor.

At another time, I made a large number of attempts at crossing on some 15 species of Campanula. Not a single hybrid was obtained. The genus can not be recommended to geneticists, therefore, though it is not too difficult cytologically, as the investigations of GAIRDNER (1926) and of DE VILMORIN and SIMONET (1927) show.

were obtained when the plants were used as female with Nos. 6 (34 pol.), 10 (29 pol.), 11 (61 pol.), 13 (24 pol.), 14 (3 pol.), and 18 (19 pol.). With No. 17, 6 capsules were obtained from 45 pollinations.

4. *L. dalmatica* Mill. Hab. Dalmatia, Oriens. Plants erect. Lvs. linear. Fls. yellow with deeper palate. Close to *L. macedonica*. Late-flowering. No flowers available for crossing with early-flowering species.

5. *L. genistifolia* Benth. Hab. southern Europe. Very similar to *L. vulgaris*, but no flowers of latter available for crossing.

6. *L. Hendersonii* (Hort.?). Similar to *L. vulgaris*, but with larger, showier flowers. Unable to find correct name or source of this type. Used as female with pollen of *L. vulgaris*, no capsules formed (8 pol.). Used as male, with *L. vulgaris*, 4 capsules were obtained out of 6 pollinations. In the latter case numerous seeds were formed containing embryos, which were probably defective, and very little endosperm. None of the seeds germinated.

7. *L. heterophylla* Desf. (*L. aparinoides* Dietr.). Hab. Mediterranean. The plants designated here were received under the name *L. aparinoides;* but they did not belong to this yellow-flowered species. Instead, they had purple flowers, with a little yellow on the palate. In all characteristics they stood close to *L. maroccana*. Used as female, no crosses were obtained with *L. Cymbalaria* (12 pol.) or *L. Broussonnetii* (*L. multipunctata*) (5 pol.).

8. *L. macedonica* Griseb. Hab. Macedonia. Broader leaves, otherwise like *L. dalmatica*. Two varieties were grown, differing only in shade of flower color. They were received under the varietal names *speciosa* and *nymphe*. Used as female, no crosses were obtained with *L. Broussonnetii* (7 pol.) or *L. reticulata* (8 pol.).

9. *L. macroura* Link. Hab. southern Russia. LINK gives no description in Enum. Hort. Berol. II, p. 137. He refers to MARSHALL (1819) Flora taurico-causica III: p. 413; but this description only designates the plant as erect and the leaves as linear and alternate. I have no idea whether the plants received under this name are true *L. macroura* or not. They have purple flowers and are very close to *L. maroccana*, though they are late-flowering. No flowers were available for crossing when the majority of the species were in flower.

10. *L. maroccana* Hook. Hab. Marocc. Erect, annual. Lvs. linear, sometimes whorled, slightly hairy. Fls. violet to reddish-violet. Used as female, no crosses were obtained with Nos. 3 (43 pol.) or 11 (13 pol.).

11. *L. Broussonnetii* Chav. (*L. multipunctata* Hoffmgg. & Link). Hab. Marocc.; Lusit. This species and the one received under the name *L. Perezii* are closely related. The yellow flowers are spotted with copper red

flecks. Used as female, no crosses were obtained with Nos. 3 (19 pol.), 10 (11 pol.), 14 (8 pol.), and 17 (9 pol.). Hybrids were obtained with pollen of *N. Perezii*.

12. *L. Pancicii* (Hort.). Apparently not *L. Pancicii* of Janka. Erect, annual. Lvs. linear. Fls. medium size, light yellow. Flowered late. No opportunity to test compatibilities with other species.

13. *L. Perezii* Gay. (Apparently is *N. Tournefortii* Steud.) Hab. southwestern Europe. Short, erect plant, apparently short-lived perennial. Lvs. narrow lanceolate, short. Fls. pale yellow. Used as female, no crosses were obtained with Nos. 3 (64 pol.), 7 (5 pol.), 8 (4 pol.), 10 (8 pol.), 14 (12 pol.), 15 (3 pol.), 17 (14 pol.), and 18 (16 pol.). Hybrids obtained with *L. Broussonnetii* reciprocally.

14. *L. reticulata* Desf. Hab. northern Africa. Erect annual. Lvs. linear, whorled. Fls. yellow with palate copper-colored; above netted with purple veins. Used as female, no crosses obtained with Nos. 3 (25 pol.), 11 (10 pol.), and 17 (19 pol.). Hybrids with *L. sapphirina* reciprocally, and with *L. maroccana* used as male.

15. *L. sapphirina* Hoffmgg. & Link (*L. delphinioides* Gay). Hab. northern Africa. Erect annual similar to *L. maroccana*. Flowers a somewhat lighter violet-rose. Used as female, no crosses were obtained with Nos. 13 (25 pol.) or 17 (11 pol.). Hybrids obtained when *L. reticulata* was used as male.

16. *L. triornithophora* Willd. Hab. Lusitan. Erect perennial. Lvs. lanceolate in groups of 3 or 4. Fls. in groups of 3; purple-striped with orange palate. Spurs inflated. Flowers late. No opportunity to test compatibilities with other species.

17. *L. triphylla* Mill. Hab. Reg. Mediterranean. Lvs. oval in threes. Flowers bright yellow. Used as female, no crosses were obtained with Nos. 3 (27 pol.), 6 (14 pol.), 10 (9 pol.), 11 (13 pol.), 13 (17 pol.), 14 (9 pol.), 18 (11 pol.).

18. *L. vulgaris* Mill. The common butter and eggs. Adv. from Europe. Erect perennial. Lvs. long, linear. Fls. yellow, darker on bearded palate.

Chromosome counts were made on several species (see HEITZ 1927). *L. Cymbalaria* has 14 chromosomes (somatic). These numbers were checked in root-tips and in buds. No lagging chromosomes. *L. heterophylla* (*L. aparanoides*) shows 6 pairs of oval chromosomes of approximately the same size at IM. No lagging. *L. macroura* shows 6 pairs of chromosomes, 4 pairs being oval and 2 pairs slightly elongated. No lagging. *L. maroccana* shows 6 pairs of oval chromosomes having approximately the same size. *L.*

Broussonnetii (*L. multipunctata*) shows 6 pairs of oval chromosomes having approximately the same size. One pair occasionally lags at IA. *L. Pancicii* shows 6 pairs of oval chromosomes of approximately the same size. One or two pairs frequently lag at IA. *L. Perezii* shows 6 pairs of chromosomes of approximately the same size. Several plates showed that one pair sometimes precedes the others to the poles. *L. reticulata* shows 6 pairs of chromosomes, of which 2 pairs are one and one-half times as long as the others. *L. triphylla* shows 6 pairs of chromosomes commonly, though several plates were counted with 7 pairs. *L. vulgaris* shows 6 pairs of chromosomes, of which one pair is somewhat larger than the others.

These results indicate that the Linaria species have six pairs of chromosomes with the exception of *L. Cymbalaria* (seven pairs). It is thus an example of a genus where marked sexual incompatibility has developed apart from changes in chromosome number. *L. Cymbalaria* has been given generic status, largely on account of its reniform-orbicular leaves. But there appears to be no valid genetic reason for such a separation. Though *L. Cymbalaria* does not hybridize with other species, the same statement may be made about several of the six-chromosome forms. Moreover, the pollen of several six-chromosome species grows normally in styles of *L. Cymbalaria;* and when the pollen of *L. triphylla* was applied, artificial parthenogenesis resulted, the plants being maternal diploids.

The four varieties of *L. Cymbalaria* cross together readily, and the resulting hybrids are fertile. The trailing character is dominant to the *globosa* or *compacta* character. The purple flowers of the common type are dominant to the white flowers of the *alba* variety.

L. sapphirina (*L. delphinioides*), *L. maroccana*, and *L. reticulata*, if the types I have are correctly designated, are simply varieties of the same species, crossing together freely in reciprocal crosses, and producing hybrids which exhibit no diminished fertility. *L. bipartita* and *L. macroura* apparently belong to the same group. This is perhaps not strange, as all of these species are found in various places around the border of the Mediterranean Sea.

The above species have genetic differences affecting flower color as follows:

P is a gene producing purple color in presence of *C*, a carminea gene hypostatic to *P*. *I* is an intensity gene. *PCI* gives a bright purple flower; *PCi* gives a pale purple flower. *V* is a gene that does not affect the intensity of color when present with *PCI* or *PCi;* but *v* with *PCI* tends to reduce the intensity of the purple color, and with *PCi* gives very light lavender-colored flowers and extends the yellow color of the palate. An additional gene, *A* (or its allelomorph) reduces the color in the spur and makes the color of the

palate a very light yellow. I was unable to determine the relationship of this pair.

Another group of species, which may be called yellow-flowered species, exhibited some sexual compatibility. The crosses *L. Hendersonii*×*L. Perezii* and *L. vulgaris*×*L. Hendersonii* set seeds which contained embryos; but the seeds did not germinate. Matings between *L. Perezii* and *L. Broussonnetii* (*L. multipunctata*) produced hybrids reciprocally. The hybrids were indistinguishable, irrespective of the way the cross was made. In habit of growth, color of flowers (with the exception of the copper-colored flecking, which was dominant), and shape and size of the leaves, the hybrids were strictly intermediate between the parental types. The hybrids were completely sterile.

One is forced to conclude that the genus Linaria will not be very useful in solving genetic problems.

LITERATURE CITED

GAIRDNER, A. E., 1926 *Campanula persicifolia* and its tetraploid form "Telham Beauty." J. Genet. **16**: 341–351.

HEITZ, E., 1927 Chromosomen und Gestalt bei Antirrhinum und verwandten Gattungen. Planta **7**: 392–410.

VILMORIN, R. DE, et SIMONET, M. 1927 Nombre des chromosomes dans les genres Lobelia, Linum, et chez quelques autres espèces végétales. C. R. Soc. Biol. Paris **96**: 166–168.

THE INHERITANCE OF VIRESCENT YELLOW AND RED PLANT COLORS IN COTTON[1]

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PREVIOUS WORK ON CHLOROPHYLL DEFICIENCIES

Deficiencies in the amount of chlorophyll in the cotton plant have been reported in relatively few instances. BALLS (1908) made a cross between an upland cotton with light green leaves and a dark green Egyptian cotton. He states that in the F_2 generation, plants with light green leaves and plants with dark green leaves appeared in a 3:1 ratio. STROMAN and MAHONEY (1925) recovered two chlorophyll deficient types in the F_2 generation from crosses between upland and Egyptian cottons (*Gossypium hirsutum*×*G. barbadense*). The first type, yellow seedlings, was due to 2 recessive genes, the segregating plants producing 15 green:1 yellow seedling. The second type was a pattern seedling character in which certain irregular areas devoid of chlorophyll on the cotyledon leaves were surrounded by normal green pigment. These pattern characters were inherited as recessives. In some cases two, in others three, pairs of genes were concerned. HARLAND (1932) also recovered from several different interspecific crosses various types which were partially deficient in chlorophyll. HORLACHER and KILLOUGH (1931) induced in upland cotton (*G. hirsutum*) by radiations a type that produces yellow seedlings lacking in chlorophyll. This yellow is lethal and a simple recessive to green, heterozygous plants producing 3 green: 1 yellow seedling. The yellow seedlings die after using up the stored food material of the seed. In all such chlorophyll deficient types which have been observed by the authors, the deficient portions are yellow due to the presence of carotinoid[2] pigments and the absence of green chlorophyll. No portion of a cotton plant devoid of carotinoid pigments was observed. The yellow seedling character mentioned above is not to be confused with the virescent yellow mature plant color discussed in this paper.

[1] Contribution from the TEXAS AGRICULTURAL EXPERIMENT STATION, College Station, Texas, Technical Paper No. 237.

[2] The authors wish to express their appreciation to Dr. R. G. REEVES, Professor of Biology, for conducting chemical and microchemical tests which show that the yellow pigment in the cotton plant is due to the two carotinoid pigments, carotin and xanthophyll.

HISTORY AND DESCRIPTION OF VIRESCENT YELLOW

The authors are indebted to Mr. R. E. KARPER for the stock of virescent yellow cotton, which was found growing on the TEXAS AGRICULTURAL EXPERIMENT STATION, Substation No. 7, Spur, Texas, in 1925. Only two plants of this kind appeared among thousands of normal green cotton plants growing in a large field of Mebane cotton. From the nature of its discovery, it would appear probable that its origin was the result of a recent mutation. The senior author has grown numerous progenies from the original material which have always bred true for the virescent yellow plant color.

The seedlings and young plants of virescent yellow cotton have a greenish yellow appearance due to a partial deficiency of chlorophyll. The yellow carotinoid pigments are not completely masked by the green chlorophyll that is present, thus giving the plants their characteristic appearance. As these plants grow older the chlorophyll increases in amount to such an extent that at maturity virescent yellow plants are less striking in appearance and not as readily distinguishable from the normal green plants. Frost gives to the virescent yellow plants a yellowish cast in the autumn.

VIRESCENT YELLOW × GREEN

In crosses we have made between virescent yellow and normal green cotton, the plants of the F_1 generation are all green. The F_2 generation segregated into 602 green:196 virescent yellow, a deviation of only 3.5 $\pm$8.26 from a 3:1 ratio. The genes concerned have been designated *V*, green, and *v*, virescent yellow.

RED LEAF COTTON

The occurrence and heredity of anthocyanin pigments in the cotton plant have been studied by several investigators. There are two general types of distribution of this pigment.

The first type is the red plant color which is produced by the anthocyanin and distributed throughout the stem and leaves making the entire plant red. This type has been described in *G. hirsutum* by MCLENDON (1912), THADANI (1921), WARE (1927, 1929), and CARVER (1929). It has also been described by LEAKE (1911) in *G. arboreum*. It is usually called red leaf cotton.

The second type is the red leaf spot which is confined to the leaf pulvinus. This spot is due to the development of anthocyanin in the epidermal and sub-epidermal cells of the petiole at the point where it divides into the leaf-veins. This type has been described by BALLS (1908, 1910) in *G. barbadense*. This red spot on the leaf is characteristic of all the so-called green

varieties of *G. hirsutum*, and of many other species of *Gossypium*. In this paper this type is classed as green leaf cotton.

The red plant color produced by the complete distribution of anthocyanin throughout the plant has been found to be a simple dominant to green plant color by each of the investigators mentioned. BALLS reports that the red leaf spot is also a simple dominant to green.

The anthocyanin pigment in red leaf cotton is distributed over the entire plant, but develops in greater quantities in those parts which are directly exposed to the rays of the sun. Less anthocyanin develops on the under sides of the branches, petioles, and leaves than on the upper sides. When these surfaces are turned over and held in position so that the sun's rays strike these under surfaces directly, they also develop as much red color as is normally characteristic of the upper surfaces. The effect of light on the development of anthocyanin pigment is further shown by the fact that plants of the red leaf strain grown in the greenhouse during the winter develop very small amounts of red pigment, giving to the plants only a slightly reddish cast on a green background, whereas plants of the same genotype grown in the field in the summer are solid red in appearance, except for the greenish underparts noted above.

When red leaf cotton is placed in an alkaline solution the anthocyanin pigment turns blue. The red color is restored when the tissue is placed in an acid solution.

RED LEAF × GREEN

Our crosses between red leaf cotton and green leaf cotton, which has the red leaf spot, have confirmed the interpretation of the inheritance of these two characters given by the investigators mentioned above. The F_1 generation from this cross was red, but of a lighter shade than the red leaf parent. The F_2 generation consisted of 357 red:122 green, a deviation of only 2.25 ± 6.39 from a 3:1 ratio.

The homozygous red (*RR*) plants can usually be separated phenotypically from the heterozygous red (*Rr*) plants. In general the *RR* plants are dark red and the *Rr* plants are light red. The genotype of any red leaf plant cannot, however, be determined definitely without a progeny test.

VIRESCENT YELLOW × RED LEAF

Virescent yellow cotton was crossed with red leaf cotton. The plants of the F_1 generation were light red. In the F_2 generation red leaf and virescent yellow plants were produced, as also were green plants and a new type which has been named bronze. These types appeared in the proportions shown in table 1.

TABLE 1

F_2 population from Virescent Yellow×Red Leaf Cotton.

	RED LEAF	BRONZE	GREEN	VIRESCENT YELLOW
Observed	256	101	94	28
Calculated, 9:3:3:1	269.44	89.81	89.81	29.94
o-c	−13.44	11.19	4.19	−1.94

p=.50

It is apparent from table 1 that two pairs of genes which are inherited independently are concerned in this cross. The genotype of the original red leaf parent was *RRVV*, that of the virescent yellow *rrvv*. The green segregates were *rrVV* or *rrVv*. The bronze segregates were due to the action of the red leaf gene *R* on virescent yellow and were of the genotypes *RRvv*, which is dark bronze, and *Rrvv* which is light bronze. All four pigments, anthocyanin, chlorophyll, xanthophyll, and carotin, are present in bronze plants and combine in such proportions as to produce this color type. Mature bronze plants are practically indistinguishable from red leaf plants. In general, the F_2 red leaf plants can be divided phenotypically into dark red and light red, and the F_2 bronze plants can be divided phenotypically into dark bronze and light bronze. However, in neither case can the separation of the genotype be made with absolute certainty without resort to the breeding test. F_3 progeny were grown from self-fertilized bolls of 40 different F_2 plants with the results given in table 2.

TABLE 2

Breeding behavior of selfed F_2 segregates.

F_2 PHENOTYPE	F_2 GENOTYPE	NO. OF F_3 PLANTS	PHENOTYPIC CLASSES IN F_3					
			DARK RED	LIGHT RED	DARK BRONZE	LIGHT BRONZE	GREEN	VIRESCENT YELLOW
dark red	*RRVV*	3	66					
dark red	*RRVv*	2	30		5			
light red	*RrVV*	1	5	21			12	1 (?)
light red	*RrVv*	11	38	92	17	34	45	12
dark bronze	*RRvv*	2			50			
light bronze	*Rrvv*	8			33	85		43
green	*rrVV*	0						
green	*rrVv*	10					174	64
virescent yellow	*rrvv*	3						35

In each case the breeding behavior of the F_2 genotype is according to expectation, with the exception of the one virescent yellow plant appearing among the progeny of the light red F_2 plant, which, according to the remainder of its progeny, must have been *RrVV*. It seems probable that this one off-type plant in the entire F_3 population of 862 was the result of mechanical mixture.

The F_3 population from selfing the light red plants heterozygous for both genes, *RrVv*, fits very closely the expected ratio of 3 dark red : 6 light red : 1 dark bronze : 2 light bronze : 3 green : 1 virescent yellow (table 3).

TABLE 3

F_3 population from RrVv Light Red F_2's.

	DARK RED	LIGHT RED	DARK BRONZE	LIGHT BRONZE	GREEN	VIRESCENT YELLOW
Observed	38	92	17	34	45	12
Calculated, 3:6:1:2:3:1	44.62	89.25	14.87	29.75	44.62	14.87
o-c	−6.62	2.75	2.13	4.25	0.38	−2.87

p=.77

The different color types of cotton plants resulting from this dihybrid cross can more readily be distinguished when the plants are bearing their first few adult leaves than at any other time in the life of the plants. It is impossible to classify such plants with any degree of accuracy at later stages of growth. Neither is classification from the cotyledon leaves accurate.

SUMMARY

1. Virescent yellow cotton, a new type, is described. This cotton is greenish yellow when young. The chlorophyll gradually increases in amount so that at maturity these plants are not readily distinguishable from normal green plants. Virescent yellow is a simple recessive to green. The genes of this pair have been designated as *V* (green) and *v* (virescent yellow).

2. Red leaf cotton is produced by the distribution of anthocyanin pigment throughout the plant. Data are presented, confirming the results secured by others, which indicate that red leaf *R* is a simple dominant to green leaf *r*.

3. Genes *R* and *V* are inherited independently.

4. The combination of *R* with *v* produces a new type named bronze. Bronze is produced by the development of red anthocyanin pigment on a virescent yellow background.

LITERATURE CITED

BALLS, W. L., 1908 Mendelian studies of Egyptian cotton. J. Agric. Sci. **2**: 346–379.

1910 Studies of Egyptian cotton. Yearb. Khed. Agric. Soc. 1909. 158 pp.

CARVER, W. A., 1929 The inheritance of certain seed, leaf, and flower characters in *Gossypium hirsutum* and some of their genetic interrelations. J. Amer. Soc. Agron. **21**: 467–480.

HARLAND, S. C., 1932 The genetics of cotton. Part VI. The inheritance of chlorophyll deficiency in new world cottons. J. Genetics **25**: 271–280.

HORLACHER, W. R. and KILLOUGH, D. T., 1931 Chlorophyll deficiencies induced in cotton (*Gossypium hirsutum*) by radiations. Proc. Texas Acad. Sci. 1931.

LEAKE, H. M., 1911 Studies in Indian cotton. J. Genetics **1**: 205–272.

MCLENDON, C. A., 1912 Mendelian inheritance in cotton hybrids. Georgia Agric. Expt. Sta. Bull. **99**: 141–228.

STROMAN, G. N. and MAHONEY, C. H., 1925 Heritable chlorophyll deficiencies in seedling cotton. Texas Agric. Expt. Sta. Bull. **333**: 20 pp.

THADANI, K. I., 1921 Some studies in heredity and variation in cotton. Thesis, Agricultural and Mechanical College of Texas.

WARE, J. O., 1927 The inheritance of red plant color in cotton. Arkansas Agric. Expt. Sta. Bull. **220**: 80 pp.

1929 Cotton breeding studies II. Heritable relationships of red plant color and leaf shape. Arkansas Agric. Expt. Sta. Bull. **243**: 38 pp.

FLEXED TAIL IN THE MOUSE, MUS MUSCULUS

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In January 1927 the writer found two albino males with markedly flexed tails. These tails were rigid at the bends, so it was evident that vertebral fusions, or at least some anatomical peculiarity other than muscular contraction, was the cause. The mutation was found in a stock which had descended from animals supplied by Dr. W. E. CASTLE of HARVARD UNIVERSITY. The abnormal males were mated immediately with normal albino females, and a flexed tailed stock was started from the flexed animals of the F_2 generation. The same mutation, or at least something resembling it in appearance, has been found in the colony several times since the original discovery.

Subsequent experience has shown that the character flexed tail is highly variable (figures 1 and 2). As a rule there are one or more permanent angles in the tail, though sometimes as many as five. They may be acute, obtuse, or right angles, and are most frequent in the proximal half of the tail, though at times one occurs near the tip. Rarely the tail turns sharply cephalad over the rump, then bends abruptly backward. This was the case in one of the two original mutant males, though, curiously enough, his numerous descendants rarely show this particular form of flexure. Curves of varying extent are sometimes found instead of the sharp angular bends, and spirals are fairly frequent. The latter are usually, but not always, near the base of the tail. They range from very tight close twists to widely open forms, and their direction is either clockwise or counterclockwise. In addition to being flexed, the tail is sometimes conspicuously shortened. The tail is usually very stiff where angles, curves, or spirals occur, and attempts to straighten it are likely to result in a break at that point. Sometimes there is no visible flexure, but palpation reveals rigid areas of varying extent. These stiff segments in straight tails may be so limited in length, and approach the normal so closely in flexibility, that considerable experience is required to decide whether or not the animal should be classified as flexed. Thus flexed tails range from extremely contorted or shortened forms to normality, and individuals show a variety of combinations of the characteristics mentioned.

Flexed tailed animals would certainly be handicapped under natural

[1] Mr. MIXTER secured the data on inbreeding. Miss PERMAR's contribution is noted in the text. Mr. HUNT planned and executed all the remaining experiments, and prepared the manuscript.

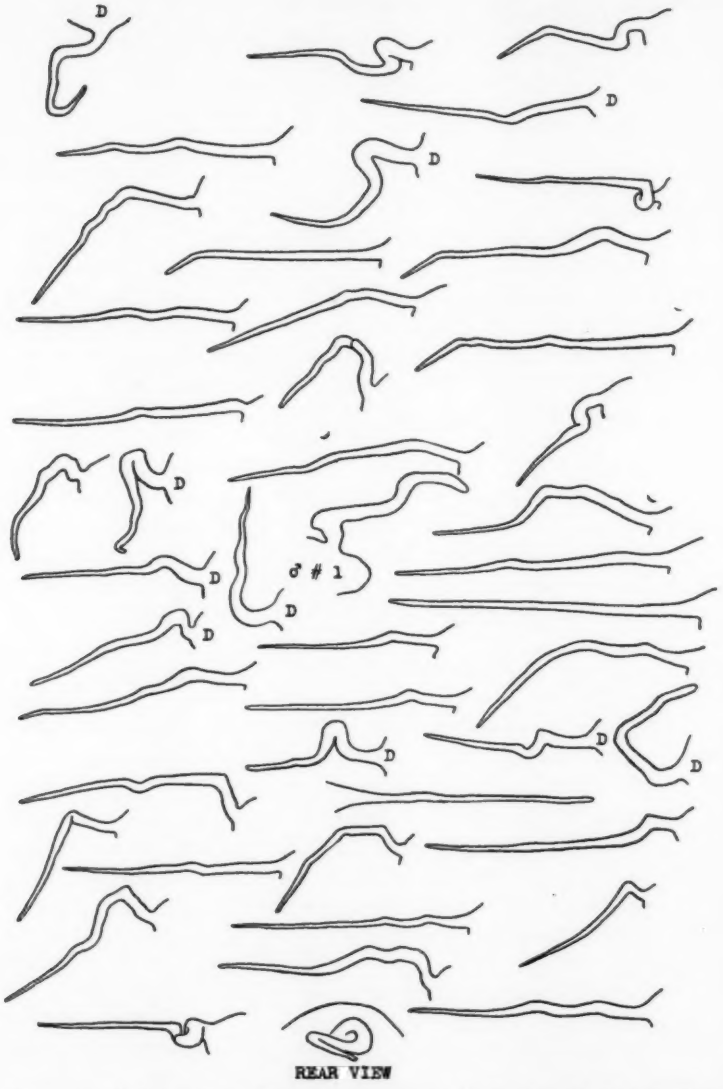

FIGURE 1.—Variations in the flexed character among closely related individuals. Male 1 (shown at the center) was mated with two normal females (crosses 1a and 1b), then with four of his resulting F_1 daughters (crosses 26a, 26b, 26c, and 26e). The flexed tails from the progeny of the latter crosses are shown in the figure. D at the base of a sketch means that it is a dorsal view, one figure gives a rear view, and all others show the right or left side.

conditions. The shredded paper bedding must be torn into short lengths, otherwise the hooked appendage of some of the mice gets hopelessly tan-

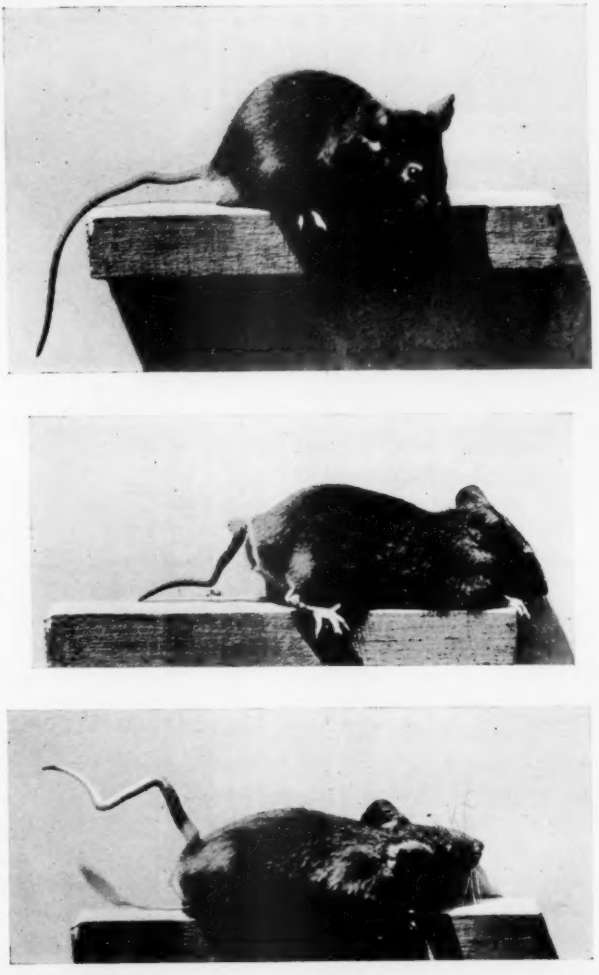

FIGURE 2.—Flexed tailed mice. The middle photograph shows marked shortening of the tail and a tendency to form a spiral at its base.

gled in it, with the result that death may come before the accident is discovered. It is evident that such extremely abnormal mice would be handicapped greatly in the wild when running from their enemies.

Several other abnormalities appeared among the flexed tailed animals. They were obviously anemic at birth. The blood was studied and the results of this investigation will appear in a later paper. The right, left, or both eyes were closed in some cases. This frequently occurred among several young from the same mating, though the exact nature of the defect and its mode of inheritance were not investigated. Some flexed tailed mice showed a dorsal enlargement of the head in front of the ears, suggesting hydrocephaly.[2] The desirability of concentrating our efforts upon the study of the complex phenomena of flexed excluded the analysis of these other traits. The eye defect particularly merits further study.

REVIEW OF LITERATURE

Several investigators have observed flexions in the mouse's tail resembling, or identical with, our mutation. PLATE (1910) studied such a character, and found it to be hereditary. BLANK (1916, 1917) investigated the embryological development and morphology of bent tail in the mouse. His material consisted of 45 mutant mice from PLATE's breed, together with 6 embryos from the uterus of a female. He found that a flexure was due to a lateral fusion between two adjacent vertebrae, which thrust the vertebral epiphyses with their growth zones, the intervertebral disc, and the vestige of the notochord toward the opposite side of the tail. The presence of a flexure demonstrated the existence of a fusion, but there might be a vertebral union without a flexure. BLANK believed that the inheritance of bent tail did not follow Mendel's Law, but his published quantitative data, as far as they go, support our contention that flexed tail is a simple Mendelian recessive character.

DOBROVOLSKAÏA-ZAVADSKAÏA has reported variations in the tail of the mouse which were encountered in experiments designed to produce changes in the germ-plasm by means of X-rays. Two types were obtained: a waltzing mouse whose trait proved to be recessive, and a dominant short tailed type which continually mutated, producing tailless, filiform tailed, kinky or bent tailed, helicoid, èt cetera, variants.

DANFORTH (1930) observed kinky tails in a strain of mice having hereditary duplication of posterior parts (extra pair of hind legs, two rectums, two urethrae, two bladders, four kidneys, four gonads, four pubic bones, doubled intestine, bifurcated spinal cord, et cetera). A genetic explanation for this kinky tail was not offered.

MISS PERMAR'S EXPERIMENTS

Miss DOROTHY PERMAR (1928) began a study of the mode of inheritance of flexed tail during the fall of 1927 in the zoological laboratory at MICHI-

[2] Since the completion of this manuscript, F. H. CLARK at HARVARD has reported on the inheritance of this hydrocephaly of the flexed tailed mouse (Proceedings of the National Academy of Sciences, vol. 18, pp. 654–656, Nov., 1932).

GAN STATE COLLEGE. Crosses were made between flexed animals to determine whether the trait breeds true. Flexed and normal mice also were mated, and the resulting F_1 generation bred F_2's.

Fourteen pairs of flexed animals produced 127 young, all of which were flexed tailed. In two additional matings one parent was stiff tailed and the other flexed; all 10 young from these two crosses were flexed. Thus the flexed tailed character bred true, like a simple Mendelian recessive, and stiff tailed animals (those in which the tail was rigid in one or more places but did not show an angular bend) bred like the flexed animals.

Thirteen crosses were made between flexed tailed and normals. In five of these crosses the male was the flexed parent and the female was normal. Eight reciprocal crosses also were bred. All the 142 F_1 generation young had normal tails. Thus it appeared that the flexed character was recessive to normality.

Thirty matings between F_1 parents produced an F_2 population of 1,065. In most of these crosses both parents had been bred by the same pair of P_1's, and frequently both the F_1's of a pair came from the same litter. Nine hundred thirty-six of the F_2's were normal tailed and 129 were flexed, a ratio of 7.26 normals to 1.00 flexed. The flexed×flexed and the flexed×normal matings gave results which were in perfect agreement with the hypothesis that flexed tail is a simple Mendelian recessive character, but the F_2 distribution was decidedly inconsistent with this view. No other satisfactory genetic explanation was found, so that Miss PERMAR'S work failed to reveal the mode of inheritance of the flexed tailed mutation.

It was obvious that a fresh start was necessary. Certain facts discovered during Miss PERMAR'S work suggested why her F_2 ratio deviated so markedly from 3:1. A flexed tail is rigid at and near the bends and sometimes the flexure is absent but the rigidity remains. Thus unless the tail were palpated it might pass for normal. Furthermore, stiffened but flexureless tails occasionally have such exceedingly short rigid segments that a hasty examination might lead one to classify them as normal. The writer has found cases in which short rigid segments can be made pliable by gently bending them two or three times between the thumb and finger. Thus the flexed character grades into normality so perfectly that great care must *always* be used in differentiating between a non-flexed but stiff tail, and one which is entirely normal. Indeed, as will be shown later, homozygous flexed animals occasionally have a tail which can not be distinguished from a normal one, so that the genetic character of the mouse can be demonstrated only by its ancestry and by breeding tests. It is therefore probable that Miss PERMAR'S deficiency of flexed tailed animals in the F_2 generation was in part due to classifying a few animals as normal which were actually flexed.

A second complicating factor was the anemia of the newborn flexed. It was discovered late in Miss PERMAR'S work that there were anemic and normal blooded mice in the F_2 litters. Mice are hairless at birth, the only color they show being the red which is due to the blood. Anemics are easily recognized by a dilution of the red color. The flexure in the tail of the mutant type can usually be observed at birth, and the newborn animals with flexed tails are anemic as a rule. It was conceivable that the anemia of the flexed tailed animals was so deleterious that their death rate during the first three or four weeks was much greater than the rate for their normal siblings. In an F_2 generation this differential death rate would give a marked excess of normals at the time of the final count, which was almost always between the twenty-first and twenty-eighth day after birth. This was the kind of result secured by Miss PERMAR. That hereditary anemia in mice can be a lethal agent was shown by Miss DE ABERLE (1927) who found that mice homozygous for the dominant white factor possess 25 percent as much hemoglobin and 14 percent as many red cells as normal mice, and that these anemics die within ten days after birth. Such considerations suggested that the flexed character could be the expression of a single recessive Mendelian factor, but that the deficiency of flexed animals in the F_2 generation might be due to classifying a few flexed mice as normals and to a higher death rate among the flexed. The writer, therefore, undertook a more critical and extensive reinvestigation of the question (HUNT 1932).

FLEXED×FLEXED MATINGS

One of the most important things to discover about a mutation is whether it breeds true. Mr. RUSSELL MIXTER attempted to isolate various grades of tail flexure in strains that would propagate true to form. The details of this experiment will be discussed later in the paper. Suffice it to say at this point that seven grades were recognized, and that a line of inbreeding was started for each. In every generation animals were mated which showed the original grade used when the line was started. The inbreeding experiment revealed, also, whether flexed×flexed crosses produced anything but flexed progeny. Forty-nine such matings yielded 688 young, of which 584 were living three to four weeks later.

Table 1 summarizes the breeding results of the flexed×flexed crosses. Eight of these were between mice whose tails were not only flexed, but also less than half the length of the body. These are referred to as "short tailed flexed." In the remaining 41 crosses the tails were of approximately normal length. The genetic behavior of the short tailed condition will be considered after noting the results for all the crosses of flexed×flexed. Six hundred forty-eight (94.2 percent) of all the young were flexed tailed

TABLE 1

Types of young bred by flexed×flexed crosses.

TYPE OF CROSS	DISTRIBUTION OF YOUNG											
	AT BIRTH					AT THE FINAL COUNTING						
	FLEXED ANEMICS	SHORT TAILED ANEMICS	DOUBTFULLY FLEXED ANEMICS	APPARENTLY STRAIGHT ANEMICS	STRAIGHT ANEMICS	FLEXED MALES	FLEXED FEMALES	SHORT TAILED FLEXED FEMALES	SHORT TAILED FLEXED MALES	SHORT TAILED FEMALES	NORMAL FEMALES	NORMAL MALES
Flexed*×flexed	574	4	16	5	2	236	256	9	1		2	3
Short tailed flexed× Short tailed flexed	74	13				31	14	23	8	1		
Totals	648	17	16	5	2	267	270	32	9	1	2	3

* The tails were of normal length.

anemics on the day of birth. All the remaining 40 young were anemic, but 17 were short tailed, 16 were doubtfully flexed, 5 were apparently straight tailed, and 2 were recorded as straight tailed.

It has been mentioned that the tail may be straight and yet have stiff segments. Thus the form at birth, at which time a stiff region would be difficult or impossible to identify, is presumably not a very reliable indication as to whether the animal is genetically flexed. The condition of the tail can be more accurately determined at twenty-one to twenty-eight days, when the final counts and classifications of the young were made. At this time 537 had flexed tails of approximately normal length. The sexes approached a 1:1 ratio. There were, in addition, 32 females and 9 males with short flexed tails, 1 female with a short tail, and 2 females and 3 males with *normal* tails. Thus the flexed×flexed crosses bred 578 flexed animals (99.0 percent) and 6 non-flexed. Practically speaking flexed breeds true, but even such a small proportion of exceptions as 1 percent should not be ignored, so Mr. MIXTER applied the breeding test to these exceptional animals.

Two of the normal males (♂125 and ♂131) produced by the flexed ×flexed crosses were mated with flexed females. The parents of these males had relatively slight flexures (grades 1 and 2). Both were anemic at birth. Male 125 mated with flexed females 123 and 124 yielded 20 offspring, all flexed at birth. Of these 20, only one survived to twenty-one days, and at that time it was found to be flexed. Male 131 paired with flexed female 132 yielded 11 young which were flexed at birth. The four alive at twenty-one days were highly flexed.

The writer has discovered several normal tailed animals in the stock cages where the flexed strain is maintained. Presumably the parents of such animals were both flexed, for we are careful to exclude foreign stock. Five such mice were found in October 1929, 2 males and 3 females. One of these males was mated with the 3 females, producing 10 normal and 17 flexed offspring. Each female bore both types of young. If the male and the 3 females be regarded as heterozygotes carrying one gene for normal tail and one for flexed, then not many more than 7 (instead of 17) of the young should have been flexed tailed. The result is explicable, however, if we assume that the grade of flexure is determined by modifying factors. In that case the normal tailed parents would be homozygous flexed, but the modifying factors would completely suppress the development of a flexure. Segregation of these modifiers would permit the flexure to appear on some of the progeny.

One of the 10 normal young mentioned in the preceding paragraphs, a male, was mated with 2 flexed females, and 8 flexed offspring were obtained. Thus this male bred like a flexed animal.

The second normal male which was found in the flexed stock cages was mated with 2 flexed females; 17 flexed offspring were bred.

It is regrettable that only 2 of the 6 non-flexed young recorded in table 1 could be tested genetically. The exceptional normal progeny of flexed ×flexed crosses merit further study. Nevertheless Mr. MIXTER's crosses, described above, strongly favor the view that such normal young are homozygous flexed animals in which flexure, or the equivalent stiffness of the tail without flexure, is suppressed by modifying factors, or by environmental agents, or by both. The same causes which inhibit flexure may be, at least in part, responsible for the great variability in bent tails. It should be remembered in this connection that some stiff tailed animals, as has already been mentioned, approach the normal condition so closely that it is difficult to classify them. The normal offspring from flexed×flexed matings may reasonably be regarded as completing the full range of variation for homozygous flexed tailed mice. This range extends, therefore, from marked kinking and extensive rigidity to a completely normal somatic condition.

When Mr. MIXTER's data on the offspring of flexed×flexed crosses are combined with Miss PERMAR's, we find that such crosses bred 715 flexed and 6 non-flexed progeny. The conclusion may be drawn that the flexed tailed character breeds true as a rule, and that the few exceptional cases are conformable with the view that there is a single gene for flexed.

Mention has been made of eight crosses of short tailed flexed×short tailed flexed. These are recorded in table 1. The tail of the short tailed mouse is less than half the length of the body. Is this short tailed condition inherited and is it caused by a gene or genes other than that for flexed?

There were 77 progeny, at the final counting, from the crosses short tailed flexed×short tailed flexed. Of these, 45 were "long tailed" and 32 were "short tailed" (41.56 ± 3.79 percent short tailed). There were 507 young from the crosses flexed×flexed where the tails of the parents were of normal length. Of the 507, 10 were short tailed ($1.97 \pm .42$ percent). Thus when both parents were flexed and short tailed, the percentage of short tailed young was 21 times as great as when the flexed parents were long tailed. The difference in percentages is statistically significant, so that the short tailed condition appears to be hereditary. Short tailed ×short tailed produced both long and short tailed progeny, and the same result was obtained when long tailed flexed animals were mated together, though in the latter case the percentage of short tailed offspring was much the lower. These facts suggest that the short tailed condition is attributable to two or more pairs of genes other than that for flexed. Probably but few short tailed animals are homozygous for short tailed genes, hence when mated together they produce a considerable percentage of long tailed

mice. On the other hand when a long tailed animal carrying some of the short genes happens to mate with another long tailed individual carrying different shortening genes, a few of the progeny will be shorts. The data, however, are not sufficient to establish the mode of inheritance of the short tailed condition. Further investigation is needed. Among other things variation in the length of flexed tails should be studied to determine whether Mr. MIXTER's definition of the short tail is purely arbitrary. Also, if short tail is not due to the gene for flexed, short tailed non-flexed strains should be built up for genetic experimentation.

FLEXED×NORMAL MATINGS (P_1'S)

If there were a single gene for the flexed character, it remained to be seen whether it was dominant or recessive to the normal straight and pliable condition of the tail. So flexed males were bred with normal females. Thirty-nine such crosses were made, and 53 litters containing 302 animals at the time of the final counting (21 to 28 days) were produced. Of these, 299 (162 males and 137 females) had normal straight tails, while 3 mice (1 male and 2 females) possessed flexed tails. These 3 flexed animals were the progeny of one female (HY15); the remaining 38 females bred normal young only.

Female HY15 produced 14 young, 12 of which survived to the time of the final count. The distribution at birth was as follows: 3 flexed anemics, 1 doubtfully flexed anemic, 8 normally red straight tailed, and 2 anemic straight tailed. The final count of her litters after weaning was 7 normal tailed males, 2 normal tailed females, 1 flexed tailed male, and 2 flexed tailed females, or a ratio of 9 normals:3 flexed. The fact that only 1 of the 39 normal females gave birth to flexed young marks her as unusual. There was something in her germ-plasm that was absent in the other mated females. She was undoubtedly heterozygous, carrying the recessive gene for flexed and its normal dominant allelomorph. The probability that this view is correct is supported by the fact that flexure has cropped out several times in different strains of our mouse colony.

Miss DOROTHY PERMAR's thirteen matings of flexed with normal tailed animals gave 142 offspring, all of them with normal tails. Adding Miss PERMAR's data to those I secured from the 38 normal females gives a total of 432 normal tailed F_1 progeny from the P_1 flexed×normal crosses. Thus flexed is recessive to the normal condition.

THE BACKCROSS (F_1×FLEXED)

The next genetic test made was to cross males and females of the F_1 generation with flexed animals. The results are summarized in table 2. Fifty-one matings were made between F_1 females and flexed males. They

TABLE 2

Distribution of the progeny of the crosses F_1×flexed.

TYPE OF MATING	NUMBER OF MATINGS	NUMBER OF LITTERS	PROGENY											
			NUMBER AT BIRTH						NUMBER AT THE FINAL COUNT					
			FLEXED TAILED		NORMAL TAILED		INTERMEDIATE OR DOUBTFUL		FLEXED TAILED		NORMAL TAILED		INTERMEDIATE OR DOUBTFUL	
			ANEMIC	NORMALLY RED	ANEMIC	NORMALLY RED	ANEMIC	NORMALLY RED	♂	♀	♂	♀	♂	♀
F_1♀ ×Flexed ♂	51	112	309	2	78	442	22	10	143	162	245	219	13	13
Flexed ♀ ×F_1 ♂	5	19	35	1	0	57	6	1	12	16	27	28	0	0
Totals	56	131	344	3	78	499	28	11	155	178	272	247	13	13
Undepleted litters from cross F_1×flexed	44	75							108	115	154	148	7	7
Undepleted litters of 7 or more animals each, from cross F_1×flexed	28	43								176		198		9

produced 112 litters comprising 863 young, of which 795 survived to the age of 21 to 28 days, when they were counted. The reciprocal cross involved 5 matings which bred 19 litters. Combining the data for the two types of crosses, the distribution of the young at birth was as follows: 344 anemic flexed tailed; 3 normally red flexed tailed (a type of considerable significance which will be discussed later); 78 anemic normal tailed; 499 normally red normal tailed; 28 anemics and 11 normally reds whose tails were of intermediate or doubtful character.

Mention may be made at this point of the frequency of anemia among these newborn mice, though we will return to the subject later. Of the 963 young, 450, or 46.73 ± 1.09 percent, were anemics. If anemia were a simple Mendelian recessive we would expect about 50 percent of the backcross generation to be anemic. The actual percentage deviates from 50 percent by 3.27 ± 1.09 percent, which is on the borderline of statistical significance.

There were 386 of the newborn which were recognizably flexed tailed, intermediate, or doubtfully flexed. This was 40.08 ± 1.07 percent of the whole. The appearance of a tail at birth, however, is an unreliable index to its real nature. One must wait until the skeletal elements are more fully formed before passing judgment, for, as has been pointed out, the tail of a partly grown mouse may be straight but as stiff in places as tails which show flexures. Also, a flexed tail may approach so closely to the normal structure that its nature is indicated in the mature mouse only by a very slight bend, or by a very short stiff segment, or both. Thus a more reliable classification of the tails can be made when the animals are three or four weeks old.

The final count was when the young were 21 to 28 days of age. The nature of the tail is usually evident at that time. There were 333 clearly flexed (155 males, 178 females), 26 of intermediate or doubtful nature (13 males, 13 females), and 519 normal tailed (272 males and 247 females). The very slightly flexed (intermediate) and doubtful cases have been combined with the obviously flexed throughout the paper in computing the percentages of flexed animals. The flexed condition grades into normality, so it seems reasonable to classify the few doubtful cases as extreme variants of flexed. The percentage of flexed animals at the final count was 40.89 ± 1.12 percent. This is not substantially different from the proportion of flexed animals at birth, but perhaps the close agreement is merely a coincidence. A considerable number of young flexed animals may have died before the final count, the percentage being maintained by the later discovery of a sufficient number of stiff but straight tailed animals to hold up the percentage.

As the investigation progressed we began to suspect that the flexed

character is semi-lethal, so that the death rate for young flexed tailed animals would be higher than for their normal siblings. If there were a recessive flexed gene, then we would find that the percentages of flexed animals in the backcross and the F_2 generations would be less than the expected 50 percent and 25 percent. Such a differential death rate could operate before birth, after birth, or during both periods. The disturbing effect of postnatal deaths can be eliminated by using only those litters which contained the same number of young at the final counting as at birth—the undepleted litters. The effect of a differential prenatal death rate on the normal:flexed ratio could be measured by determining the number of zygotes formed, then using only the litters which had been undepleted by death within the uterus. This would have involved counting the corpora lutea in the pregnant females. Since this involves a time-consuming and somewhat difficult technique we did not attempt it. However, the disturbance caused by a prenatal differential death rate can probably be reduced by using only the *large* postnatally undepleted litters, and this we did, as will appear shortly.

There were 44 matings, producing 75 undepleted litters with a total of 539 young. As stated previously an undepleted litter was one in which no deaths occurred between birth and the final counting of the litter when it was three to four weeks old. The distribution of the young in these litters is shown in table 2. If, as previously done, we class the 14 intermediate or doubtful cases with the flexed category, the percentage of the latter rises to 43.97 ± 1.44 percent. Thus postnatal deaths decreased the percentage of flexed animals by about 3 percent.

The postnatal death rate among flexed animals in the 131 unselected litters was apparently rather high. 43.97 percent of the young in the undepleted litters were flexed tailed. This percentage is based upon 539 animals and is therefore quite reliable. There were 963 on the day of birth in the unselected litters. If none of these young had died, there should have been about 423 flexed animals at the final count when they were 3 or 4 weeks old ($963 \times .4397$). There were, however, 359 flexed young at this time, indicating that around 64 such animals must have died. This is a death rate of about 15.13 percent. Using these data, the computed death rate among the straight tailed young of the unselected litters was 3.89 percent. Thus deaths among the flexed were nearly four times as frequent as among normals, proving our contention that there is a differential death rate in mixed litters. This matter will be considered again in connection with the F_2 generation.

We used those undepleted litters which contained 7 or more animals each to eliminate as far as possible the complications arising from a prenatal, in addition to the postnatal, differential death rate. There were 43

such litters from 28 matings (table 2). These litters were probably fairly highly selected for a low prenatal death rate. The fact that they suffered from no postnatal deaths whatever suggests that they were relatively free from hereditary weaknesses, and that the mothers were in good health during gestation as well as when nursing. Such conditions should have reduced the prenatal deaths. Also, selecting litters of average size (7) and larger undoubtedly eliminated some in which a considerable proportion of the fetuses died.

If using these large undepleted litters eliminated all prenatal deaths, and if there is a single recessive flexed gene present in all the F_1's, then these backcross litters should have contained approximately 50 percent of mutant animals. It is unlikely that there were no prenatal deaths, but they were probably few, and in this case nearly half the young should be flexed if there is a recessive gene for flexure. A hypothetical example will illustrate the point.

Suppose 2,000 zygotes are formed, half of them heterozygous for flexed and half homozygous. Assume that the prenatal death rate is low, say 5 percent. There will be 100 deaths. If, as in the first two or three weeks after birth, about four times as many flexed as normal zygotes die, then approximately 80 of these dead fetuses will be flexed and 20 normal. Thus at birth the litters will contain 920 flexed and 980 normals, and the percentage of the former will be 48.42 percent.

Let us now turn to the facts. The 43 undepleted litters containing 7 or more each produced 383 animals, of which 176 were flexed, 198 normals, and 9 had tails which were intermediate or of doubtful nature. If we include the 9 intermediate or doubtful cases with the flexed, the percentage of these in the backcross litters becomes 48.30 ± 1.72 percent. This is in close agreement with what one might expect with a low prenatal death rate, considerably higher among the flexed than the normals, if there is a recessive gene for flexed.

The above is 7.41 ± 2.05 percent higher than the frequency of flexed (40.89 ± 1.12 percent) in the unselected litters. This difference is 3.6 the size of its probable error and is therefore statistically significant.

The possible rôle of modifying factors should be mentioned again. Some of the variability in flexed animals is no doubt due to such genes which sometimes are so potent as to suppress the character in a homozygote. A part of our flexed deficiency in the backcross generation may be the work of these modifiers, though one would not expect them to be so numerous and their collective effect so potent in the backcross as in the F_2 generation, because the backcross progeny had both a flexed parent and grandparent, while the F_2 animals had only flexed grandparents.

GENETIC CONSTITUTION OF THE STRAIGHT TAILED PROGENY OF THE $F_1 \times$ FLEXED CROSSES

If there is a recessive gene for flexed, then the normal young produced by the $F_1 \times$ flexed crosses should be heterozygous for flexed, and should therefore breed like F_1's. The constitution of these normal tailed mice can be tested by crossing them with flexed animals. If *F* be the symbol for the normal gene, and *f* for the flexed, then the following describes the process:

P_1 generation: $FF \times ff$
F_1 generation: all Ff
Backcross: $Ff \times ff$
Progeny of the backcross: Ff (normal) + ff (flexed).

If the *Ff*, normal tailed, offspring of the backcross were mated, they should give practically the same results as though the F_1's were substituted for them. Such an experiment was carried out by crossing 76 normal tailed female offspring of the $F_1 \times$ flexed matings with flexed males. The results are summarized in table 3, where comparisons are made with the $F_1 \times$ flexed matings. Seventy-nine litters were secured. As a rule only one litter was bred by each female. The primary object of the experiment was at first to determine whether any of these somatically straight tailed females were homozygous for the flexed factor. Therefore, as soon as a female was found to produce both normal and flexed young she was no longer used.

TABLE 3

Comparisons between the progeny of the $F_1 \times$ flexed crosses and the progeny of the crosses (straight tailed female offspring of $F_1 \times$ flexed) $\times$ flexed.

PERCENTAGE OF:	PROGENY OF:	
	$F_1 \times$ FLEXED	(NORMAL TAILED FEMALE OFFSPRING OF $F_1 \times$ FLEXED) $\times$ FLEXED
Flexed tailed young at birth	40.08 ± 1.07	44.07 ± 1.54 (472 young)
Flexed tailed young at the final counts	40.89 ± 1.12	39.07 ± 1.67 (389 young)
Flexed tailed young in undepleted litters	43.97 ± 1.44	43.01 ± 2.45 (186 young)
Flexed tailed young in undepleted litters where the litters contained 7 or more young	48.30 ± 1.72	47.06 ± 4.08 (68 young)
Anemic young at birth	46.73 ± 1.09	44.91 ± 1.58 (452 young)
Anemic young at birth in litters of 7 or more animals	47.21 ± 1.29	45.88 ± 2.41 (194 young)

The breeding behavior of these normal tailed females closely resembled that of the F_1's. The normal daughters of the $F_1 \times$ flexed crosses when

mated with flexed males produced 472 young, of which 44.07 percent were recognized as flexed at birth. The corresponding figure for the progeny of $F_1 \times$flexed experiments was 40.08 percent. When the final counts were made at 21 to 28 days, 39.07 percent of the 389 young from the normal tailed females were found to be flexed as compared with 40.89 percent for the backcross progeny. Undepleted litters (those in which there were no deaths between birth and the final count) yielded about 43 percent of flexed animals in both experiments. Undepleted litters of 7 or more young contained 48.30 percent of flexed animals in the backcross experiment, and 47.06 percent in the other. The frequency of anemia also was about the same in the two series. The percentage of flexed animals in the progeny of the $F_1 \times$flexed matings is not significantly different in any of the six comparisons from the percentage of flexed among the offspring of the normal females produced by the $F_1 \times$flexed pairs. This is obvious when one inspects the differences and probable errors in table 3.

The normal tailed daughters of $F_1 \times$flexed crosses therefore breed like F_1 animals, and this is exactly the result to be expected if there is a recessive flexed gene. The data of table 3 therefore furnish one more link in the chain of evidence that flexed is a simple recessive Mendelian character.

Mention has been made of the fact that the original purpose of this experiment was to determine whether somatically normal tailed animals might not be homozygous for flexed. Environmental factors in embryonic development might, conceivably, prevent permanent fusions between caudal vertebrae. If such an event occurred fairly frequently, the result would be a deficiency of flexed animals such as was found in the backcross litters. Since flexed $\times$ flexed matings yield almost nothing but flexed young, a homozygous flexed female whose tail appeared normal might be expected to produce none but flexed tailed offspring when mated with a flexed male. As a matter of fact only one of these 72 females that gave birth to litters had only flexed young; all the remaining 71 produced both flexed and normals. The exceptional female gave birth to 6 flexed animals in one litter, 3 of which survived. She had two small litters later, but all these infants were born dead. This female may have been heterozygous for flexed rather than homozygous, for the production of 6 flexed young when a heterozygote is bred with a flexed mate should occur about once in 64 times. Somatically normal but genetically homozygous flexed mice can not be numerous enough to account for all the shortage of flexed animals in our experiments.

THE F_2 GENERATION

Miss DOROTHY PERMAR, as has been mentioned, found an F_2 ratio of 7.26 normals:1.00 flexed. This ratio conforms to no simple Mendelian

mechanism of inheritance. As Miss PERMAR'S work progressed several sources of error became evident which, it was thought, might account for the marked deviation from a 3.00:1.00 ratio. For example, it is probable that some straight tailed animals having stiff caudal segments were erroneously classified as normals. The practice of palpating the tail to locate stiffened sections began after the investigation was well advanced. Also, it was not realized at the outset that extreme variants of the flexed can scarcely be distinguished from normal tailed mice. This fact, too, was brought out when we began to palpate carefully all apparently normal tails in generations that produced both flexed and normal animals. So the writer repeated the experiment, starting from the beginning with fresh pairs of P_1's.

Three flexed tailed males were mated with normal females from our laboratory stocks. Each male was confined in a cage with 6 females, so that there were 18 P_1 crosses in all. The F_1 generation has already been discussed. The distribution of the F_2 young is shown in table 4. Forty-four F_1 matings bred 206 F_2 litters containing 1478 young on the day of birth. The condition of the tail in some of these new-born mice was, as in the previous experiments, difficult to determine, so that a considerable number were classified as "intermediate or doubtful." Whether the animal was normal blooded or anemic was likewise problematical in over 2 percent of the cases. If the intermediate or doubtful cases are combined with those that were certainly flexed, there were 289 flexed and 1189 normal tailed, a ratio of 4.11 normals:1.00 flexed, or 19.55 ± 70 percent flexed. Three hundred and ten of the new-born mice were certainly anemic and 1136 clearly normal blooded, as judged by the redness of the mouse. Thus there were 3.66 normally red animals:1.00 anemic, or $21.44 \pm .73$ percent of the young, exclusive of the doubtful cases, were anemics.

The discussion of the backcross experiments brought out the fact that the classification of tails soon after birth can not be very reliable because the only criterion for flexure at that time is external appearance. The distribution of F_2's at the final count (when the mice were 21 to 28 days of age) is shown in table 4. We assume that the intermediate or doubtful cases were usually extreme variants of flexed in the direction of normality. Combining them with the flexed, there are 213 flexed and 1104 normal tailed animals, which make a ratio of 5.18 normals:1.00 flexed, and 16.17 $\pm .68$ percent of flexed tailed mice. This is a wide departure from the 25 percent for a Mendelian character in an F_2 generation, so it is necessary to determine whether here also a higher death rate among the flexed than for the normals may have caused a deficiency of flexed.

Table 4 gives the distribution of animals in undepleted F_2 litters, by which is meant those litters which suffered no mortality at all between

TABLE 4

Distribution of the F_2 generation.

	NUMBER OF F_1 MATINGS	NUMBER OF F_2 LITTERS	PROGENY															
			NUMBER AT BIRTH									NUMBER AT THE FINAL COUNT						
			FLEXED TAILED			NORMAL TAILED			INTERMEDIATE OR DOUBTFUL			FLEXED TAILED		NORMAL TAILED		INTERMEDIATE OR DOUBTFUL	
			ANEMIC	NORMALLY RED	CONDITION OF THE BLOOD UNCERTAIN	ANEMIC	NORMALLY RED	CONDITION OF THE BLOOD UNCERTAIN	ANEMIC	NORMALLY RED	CONDITION OF THE BLOOD UNCERTAIN	♂	♀	♂	♀	♂	♀
All F_2's	44	206	181	2	11	81	1097	11	48	37	10	77	109	545	559	12	15
F_2's of undepleted litters	39	114										57	70	339	314	6	9
F_2's of undepleted litters of 7 or more animals each	34	73										43	59	250	234	5	4

birth and the final counting at three to four weeks of age. Thirty-nine F_1 matings produced 114 F_2 undepleted litters containing 795 animals. Of these, 127 were certainly flexed and 15 intermediate or doubtful, making 142 to be reckoned as flexed if we follow the plan of regarding as flexed all animals that are not clearly normal tailed. There were 653 normal mice, which gave an F_2 ratio of 4.60 normals:1.00 flexed, or $17.86 \pm .92$ percent of flexed tailed mice in the undepleted F_2 generation. This was an increase of 1.69 percent over the percentage of flexed for the entire F_2 generation (depleted plus undepleted litters), but the increase was not statistically significant, though it is important to note that when all possible disturbing effects of a differential postnatal death rate were removed, the percentage of F_2 flexed animals moved toward 25 percent.

The attempt was then made to eliminate not only the postnatal, but at least part of the prenatal deaths as well. It will be recalled that the same object was sought in analyzing the backcross generation. Table 4 gives the types of young in undepleted litters which contained 7 or more animals each. There were 34 such F_1 matings which bred 73 F_2 litters containing a total of 595 young. There were 102 clearly flexed tailed, and 9 intermediate or doubtful animals, making a total of 111 which we reckon as flexed, while the normal tailed numbered 484. The F_2 ratio for these animals was 4.36 normals:1.00 flexed. The percentage of flexed animals rose to 18.66 ± 1.08 percent, which was an increase of 2.49 ± 1.28 percent over the $16.17 \pm .68$ percent of flexed animals for the whole unselected F_2 population. This difference was only 1.9 times its probable error. Even though eliminating all of the postnatal and part of the prenatal deaths did not cause a statistically significant increase in the percentage of flexed animals, yet there was an increase of noteworthy size, and to this extent the facts lend support to the view that a differential death rate is one of the causes for the frequency of flexed animals being considerably below 25 percent.

The probable effects of the differential death rate are shown by the following computations. There were 17.86 percent of flexed animals in the undepleted F_2 litters at the final count. It is possible that if all the 1478 F_2 young, that is all the mice born in the F_2 generation, had survived to the age at which the animals were finally counted (3 to 4 weeks), about the same percentage of flexed mice would have been found among them. In other words there would have been approximately 264 ($1478 \times .1786$) flexed tailed individuals. There were actually only 213, so that something like 51 flexed animals (264–213) probably died. This was a death rate of 19.32 percent for the young flexed animals. If there were 264 flexed young among the newborn, then there were 1214 normal tailed animals at that time. This number had fallen to 1104 when the young were 21 to 28 days

old, a decrease by death of 110 animals, or a death rate of 9.06 percent. Thus it appears plausible that the mortality rate was about twice as great among the animals with the defective tails as among the normals.

Possibly the undepleted litters were selected to some extent for a low percentage of flexed animals. If flexed animals have a higher death rate than normals, then there is more likelihood of deaths occurring in litters having a relatively large number of flexed animals than in litters having fewer flexed. Thus it may be that if no deaths had occurred among our whole F_2 population, we would have found considerably more than 17.86 percent of flexed mice at the final count.

The data suggest modifying factors. When death rates were materially reduced the percentage of flexed animals in the F_2 generation fell below 25 percent by 6.34 percent, while in the backcross generation (table 3) the percentage of flexed was only 1.70 percent less than the expected 50 percent, and among the progeny of matings between flexed males and normal tailed daughters of the $F_1 \times$flexed cross the percentage of flexed was only 2.94 percent less than 50 percent. The hypothesis of modifying factors fits the facts fairly well. The F_2 flexed animals all had 2 flexed grandparents and 4 flexed great-grandparents. The progeny of the $F_1 \times$flexed crosses had a flexed parent, 3 flexed grandparents, and 6 flexed great-grandparents. The young of the matings between flexed males and normal daughters of the $F_1 \times$flexed crosses each had one flexed parent, 3 flexed grandparents, and 6 flexed great-grandparents. A greater concentration of flexed tailed germ-plasm is thus associated with a closer approximation to the percentages one should get if flexed is a simple recessive. This fact supports our theory that flexed is such a character, but that other genes, which are few or absent in decidedly flexed animals, may modify the character, or even suppress it altogether if enough of them are present. The conception of modifying factors finds further support in the fact that flexed is a highly variable trait which ranges all the way from very extreme forms to normality itself.

The reader has probably noted the deficiency of males among the flexed animals recorded in the tables. The progeny of the following crosses were classified by sex and condition of the tail to compare the sex ratios among flexed and normals: flexed $\times$ flexed; flexed $\times$ normal; $F_1 \times$ flexed; $F_1 \times F_1$; and normal female (bred by the cross $F_1 \times$ flexed) $\times$ flexed male. These crosses produced a total of 585 flexed males, 656 flexed females, 1097 normal males, and 1062 normal females. Thus $47.14 \pm .96$ percent of the flexed animals were males, while among the normal mice the percentage was $50.81 \pm .73$ percent. There was thus a slight deficiency of males in the flexed category. The difference, 3.67 ± 1.21 percent, scarcely exceeded three times its probable error, so that it was of doubtful significance. However,

the males were decidedly in the minority among the flexed animals bred by three of the four crosses mentioned above, so there are grounds for suspecting that there is some factor (or factors) among flexed mice that modifies the sex ratio and that such an influence is not found among normal animals. Perhaps flexed males have a higher death rate than flexed females.

INHERITANCE OF THE GRADE OF FLEXURE

The fact has been emphasized that the flexed character is highly variable. There are spirals, tails with one or more angles of varying magnitude, straight tails with stiff segments, and finally occasional individuals that appear normal. Variability is of the continuous type. It was shown earlier in the paper that abnormal shortness of the tail in flexed mice is probably inherited. If this modification is determined by genes other than the flexed gene, the suspicion is aroused that perhaps all variations in flexure are more or less hereditary. This possibility was tested experimentally.

Before such a study could be undertaken it was necessary to devise a system for classifying the degrees of flexure. Since variability is strictly continuous and sharp natural boundaries between different grades do not exist, the system was necessarily somewhat arbitrary. Three fundamental characteristics could be used: (1) length, (2) the percentage of the length which was rigid, and (3) the number and magnitude of the angles. The short tailed condition has been shown to be probably hereditary, so it need not be considered further. The system of grading adopted was based on the number and size of the angles, and upon stiffness where angularity was absent or slight. Other, perhaps equally valid, criteria could have been used.

The scheme for defining the different grades was as follows:

Grade 1. No, or almost no, visible flexure. Stiffness might easily be detected in the tail when it was bent a little and passed between the thumb and index finger, or the stiffness might be slight. Doubtful cases were included in this group, that is those in which flexure, or stiffness, (or both) was so slight that it was difficult to classify them with certainty either as normal or flexed animals.

Grade 2. Characterized by one slight but well defined flexure in the tail.

Grade 3. Two or more slight flexures.

(A "slight" flexure was arbitrarily defined as one at which the distal segment was deflected by 30° or less from the axis of the adjoining proximal segment. If this angle was greater than 30° the flexure was "pronounced".)

Grade 4. One pronounced flexure.

Grade 5. Two or more pronounced flexures.

Grade 6. The flexure assumed the form of a spiral—a "corkscrew tail."

If the type or degree of flexure is inherited, that is, if there are factors other than the flexed gene itself which determine the form of the tail, then one might expect the following: (1) The flexed animals in the F_2 generation from a P_1 cross of flexed×normal might have tails resembling that of the flexed P_1 ancestor; and the same thing could be true of the flexed individuals produced by a cross between F_1's and a flexed, providing the flexed parent and grandparent were of the same, or nearly the same, grade of flexure. It is conceivable, of course, that in such experiments genes from the normal P_1's might neutralize the effects of specific modifiers from the flexed ancestry. (2) If close inbreeding were carried on within each of the grades of flexure defined above, strains should be secured which would breed approximately true for the grade in question. (3) Finally, there should be a positive correlation between the grades of parents and offspring.

Each of these three tests for modifiers was applied. The F_2 flexed animals derived from the P_1 crosses flexed ♂×normal ♀ were graded by the system for tail classification just outlined. There were 207 F_2 flexed mice which descended from three mutant P_1 males, all of grade 5. The distribution of these flexed F_2's was as follows:

GRADE OF FLEXURE	1	2	3	4	5	6
Number of F_2 flexed mice	34	37	29	55	41	11

The above data seem to indicate that the degree of flexure is inherited, for the mode was at grade 4, and the second largest class was grade 5, while the flexed grandfathers were of grade 5. Such a conclusion is not to be drawn, however, without further investigation, for as will be shown shortly by the results of breeding mutants with one another, the modal grade among the offspring was 5, whether the parents showed a high or a low grade of flexion. Thus P_1 parents of grades 2 or 3 might have had F_2 descendants whose mode was at 4 or 5. An explanation will be offered later for this peculiarity in the inheritance of flexed tail.

The effect of the flexed ancestry was then determined on the grade of the backcross progeny from matings between F_1 females and flexed males. Since only those matings were used in which the same flexed male was both father and grandfather of the litters produced, or the father and grandfather were of the same grade of flexure, the number of young available was limited to 161. The data are shown in table 5.

TABLE 5

FLEXED MALE ANCESTORS		GRADES OF BACKCROSS FLEXED OFFSPRING						MEAN GRADES OF OFFSPRING
FATHER	GRANDFATHER	1	2	3	4	5	6	
		NUMBERS OF OFFSPRING						
6	6 (grade 3)	9	8	7	20	30	8	3.95
5	5 (grade 4+)	1			3			3.25
1	1 (grade 5)	2	1	3	5	8		3.84
26 (grade 4)	5 (grade 4+)	1	2	2	6	2	2	3.80
27 (grade 5)	7 (grade 5)	2	2	5	15	13	4	4.15

Mean grade of offspring where the flexed ancestor (♂6) was of grade 3: 3.95
Mean grade of offspring where the flexed ancestors (♂5 and ♂26) were of grade 4 or 4+: 3.68
Mean grade of offspring where the flexed ancestors (♂1, ♂7, and ♂27) were of grade 5: 4.05

It is obvious that table 5 furnishes no satisfactory evidence that the grade of flexure is influenced by genes, though the normal blood in these flexed animals may have prevented such factors from manifesting themselves.

Close inbreeding was finally used to determine whether the grade of flexure was inherited. Mr. RUSSELL MIXTER collected all the data in this part of the investigation. His inbreeding experiments began late in June 1929, and continued until July 1930. If genetic factors determine the extent of flexion, then by mating animals of about the same grade, selecting siblings of this grade from among their progeny and mating them together, and continuing this process for several generations, one should be able, in some lines at least, to evolve a strain of flexed mice which varies within relatively narrow limits around the grade selected in each generation. This would be due, of course, to increasing homozygosity.

Mr. MIXTER started this experiment with 32 females, representing all the grades of flexure which have been described, each female being mated with a male of her own kind. Table 6 shows the numbers of animals,

TABLE 6

Numbers of individuals mated in successive generations of the inbreeding experiment.

GENERATIONS	GRADES OF INDIVIDUALS MATED						
	1	2	3	4	5	6	SHORT TAILED
First	1♂,5♀	1♂,2♀	1♂,5♀	1♂,5♀	1♂,5♀	1♂,5♀	1♂,5♀
Second	1♂,2♀	1♂,1♀	3♂,4♀	4♂,7♀	7♂,15♀	2♂,3♀	2♂,4♀
Third	2♂,2♀				6♂,10♀		2♂,3♀
Fourth							1♂,1♀

classified by grades, used in the first and succeeding generations. Five females having a tail flexure of grade 1 were mated with a single male

of the same grade. The matings for grades 3, 4, 5, 6, and the short tailed type each consisted, likewise, of 5 females and 1 male. Two grade 2 females were bred with a single male of that type. Thus there were 32 crosses in the first generation. It has been emphasized that the classification of the grades of flexure is arbitrary, that these grades are not separated from one another by natural gaps, but that variability is continuous. Perhaps the short tailed type shows fewer intergrades than any of the others. Mr. MIXTER defines it as having a tail which is less than half as long as the body.

The mating cages of the inbreeding experiment were inspected twice a week to discover pregnant females, which were isolated. The litters were observed every three days to make note of deaths. The young animals were weaned and usually classified when 21 to 25 days old, and one or two drawings of each tail were recorded. From among the offspring, mice of the same grade of flexure as the parents were chosen to breed a new generation. The members of the first generation were selected from our stock cages, and were but remotely, if at all, related, but in later generations the matings were always between siblings.

Mr. MIXTER was unable to develop pure lines of flexed animals because breeding practically ceased in all seven lines in the second or third generation. Several difficulties were encountered. Sometimes he obtained males whose tail structure made them eligible for breeding, but no sisters of the same grade were born, and *vice versa*. Our laboratory stock of flexed animals seemed, on the whole, to propagate less rapidly than normal mice, so only what appeared to be healthy animals were selected for inbreeding, and for this reason fewer matings than possible were made. The experiment automatically terminated at the third generation, for the third generation animals practically ceased breeding. Whether this was due to increasing sterility, to miscarriages, or to the females' inability to bear young as a consequence of malformations of the pelvic girdle, we do not know. The harmful effects of this limited course of inbreeding, combined with the inferior reproductivity of the flexed stock, are probably sufficient to account for this result. The inbreeding must have been an important factor in suppressing reproduction, for only one of the 32 females of the first generation was sterile.

In spite of the fact that the inbreeding experiment failed to establish homogeneous lines of mice, it showed that there are probably genes which cause variation in the degree of flexure. This is brought out by inspection of the distribution of young from the different types of crosses and by the correlation between parents and offspring. The matings used in MIXTER'S experiment were obviously superior to the F_2 and backcross material for determining whether the degree of flexure is inherited. When a flexed ani-

mal is mated with a normal one, the F_2 flexed descendants might conceivably receive modifying genes not only from the flexed P_1 ancestor, but from the normal one also. The genes from the latter might partly or completely neutralize the effects of modifiers from the flexed P_1, so that the resemblance between the P_1 and F_2 flexed mice would be very much reduced. The same criticism would apply to a lesser extent if a correlation be sought between the flexed animals of a backcross generation (produced by $F_1 \times$ flexed matings) and their flexed ancestors.

Table 7 is a summary of Mr. MIXTER'S inbreeding work. The left half of the table shows the results of mating the first generation animals, which were selected from the stock cages. The right half records the consequences of inbreeding the second generation. Table 7 reveals some very interesting facts. No grade of flexure bred true; in fact if the offspring of the first and second generation crosses are combined, it is found that in four of the seven types of matings (grades 1, 2, 4, and 5) the progeny included all grades of tail flexure. Five hundred and sixteen animals constituted the second and third generations, and of these, 248 (48.1 percent) were classified in grade 5. This conspicuous placement of the mode at 5 was found among the offspring of all the types of crosses where the numbers were fairly large. The heaping up of nearly half the young in one grade suggests that it may have included more genotypes than any of the others, and that more than one grade might well have been created from grade 5.

The relatively high percentage of short tailed progeny from crosses of short tailed animals is conspicuous. The short tailed crosses listed in table 7 produced 58 young, 23 of which were short tailed (39.7 percent). Among the 458 young bred by all the other crosses, only 16 (3.5 percent) were short tailed, and 12 of these were the offspring of the grade 5 cross. As has been previously stated, the evidence indicates that the short tailed condition is inherited.

Tables 8 and 9, particularly the latter, indicate that hereditary factors are involved in determining the extent of flexure. Table 8 shows the mean grades of the inbred flexed offspring produced by different grades of parents, the short tailed parents and their progeny being excluded. Parents of grade 1 bred 74 individuals having an average grade of 4.01. The rating of the offspring rose slightly and irregularly as the level of the parents increased to 6.

More striking evidence of factors modifying the degree of flexure is found in table 9, where parents and offspring are classified as "low grade" (grades 1–3) and "high grade" (grades 4–6). 84.9 ± 1.5 percent of the offspring of "high grade" parents were themselves "high grade," while only 65.2 ± 2.4 percent of the young of "low grade" parents were "high grade." The "high grade" parents thus produced 19.7 percent more "high grade"

TABLE 7

Distribution of offspring in the inbreeding experiment.

GRADES OF THE FIRST GENERATION	GRADES OF THE OFFSPRING OF THE FIRST GENERATION (SECOND GENERATION)								GRADES OF THE SECOND GENERATION WHICH WERE USED AS PARENTS	GRADES OF THE OFFSPRING OF THE SECOND GENERATION (THIRD GENERATION)							
	1	2	3	4	5	6	SHORT	TOTAL		1	2	3	4	5	6	SHORT	TOTAL
1	7	3	8	9	37	4	1	69	1	2	1	2		1			6
2	4	7	7	3	21	1	1	44	2			1	1	2		1	5
3	3	2	17	11	25	2		60	3					2	1		3
4	4	2	10	16	42	11	1	86	4				1	3			4
5		1	6	14	49	9	11	90	5	1	1	4	12	28	4	1	51
6		2	8	4	15	11		40	6								0
Short			3	5	14		11	33	Short		1	1	1	9	1	12	25
Total	18	17	59	62	203	38	25	422	Total	3	3	8	15	45	6	14	94

progeny than did "low grade" parents, and this difference can scarcely have been due to random sampling, for it was seven times as large as its probable error. Thus it is evident that though the more extreme degrees of flexure did not breed true, yet they produced a higher percentage of markedly flexed offspring than the less pronouncedly flexed parents.

TABLE 8

Average grades of the inbred flexed tailed offspring of the second and third generations produced by the different grades of parents. The short tailed parents and offspring are excluded.

GRADE OF PARENTS	AVERAGE GRADE OF OFFSPRING
1	4.01 (74 individuals)
2	3.81 (47 individuals)
3	4.05 (63 individuals)
4	4.46 (89 individuals)
5	4.67 (129 individuals)
6	4.63 (40 individuals)

TABLE 9

Distribution of offspring in relation to the high or low grade of the parents.

GRADES OF THE PARENTS OF THE SECOND AND THIRD GENERATIONS		GRADES OF THE SECOND AND THIRD GENERATIONS			
		LOW GRADE (1–3)		HIGH GRADE (4–6)	
		NUMBER	PERCENTAGE	NUMBER	PERCENTAGE
Low grade crosses	1	23	31.1	51	68.9
	2	19	40.4	28	59.6
	3	22	34.9	41	65.1
High grade crosses	4	16	18.0	73	82.0
	5	13	10.1	116	89.9
	6	10	25.0	30	75.0
Totals		103		339	

Percentage of high grade offspring from high grade parents:	84.9±1.5 percent
Percentage of high grade offspring from low grade parents:	65.2±2.4 percent
Difference:	19.7±2.8 percent

Further evidence that the type of flexure is to some extent determined by hereditary is found in the data of table 10 where the grades of the parents are correlated with those of the offspring. These statistics too were derived from MIXTER's experiment on inbreeding. There were 39 matings which produced 487 young. In 31 of the matings both parents had the same grade of flexure; in 8 (21 percent) the two parents were of different grades, 5 pairs differing by one (2 and 1; 2 and 3; 3 and 4; 4 and 5), and 3

pairs by two grades (2 and 4; 3 and 5). Whenever the two parents were of different grades, the average of their ratings was used in table 10. This accounts for the parental grades $1\frac{1}{2}$, $2\frac{1}{2}$, $3\frac{1}{2}$, $4\frac{1}{2}$, and $5\frac{1}{2}$. Seventy-nine (16 percent) of the young were the progeny of such crosses. The coefficient of correlation between parents and offspring was $+.243 \pm .029$, a rather low, but nevertheless statistically significant correlation.

TABLE 10

Correlation between the grades of flexure of parents and offspring.

		GRADES OF FLEXURE OF OFFSPRING					
		1	2	3	4	5	6
GRADES OF FLEXURE OF PARENTS	6		2	8	4	15	11
	$5\frac{1}{2}$						
	5	3	3	12	28	97	17
	$4\frac{1}{2}$	1	1	2	10	21	8
	4	6	2	10	19	30	6
	$3\frac{1}{2}$					2	1
	3	1	2	12	9	30	
	$2\frac{1}{2}$			1	1	2	
	2	2	4	4	2	9	1
	$1\frac{1}{2}$	1	1	3	9	15	
	1	10	6	7	5	27	4

$n=487$; $r=+.243 \pm .029$; ratio=8.4.

The mean was calculated for the parents of each grade of offspring. These six averages when plotted were found to be approximately linear, so that the coefficient of correlation may properly be used to measure the resemblance between parents and offspring. However, only about 6 percent (the square of the coefficient of correlation, $.243^2 \times 100$) of the variability among the offspring can be definitely attributed to the parents. This coefficient of correlation should not be regarded as an exact measure of hereditary influences modifying the expression of flexure, for another system of classifying the tails (by the number of fused vertebrae, for example) might have given a considerably different value for r. Assuming that the stiffened condition of the tail is due to the fusion of vertebrae, it is reasonable to suppose that movements of the fetus and uterus would at critical periods in the development of the character modify the angle of flexure or break up incipient adhesions between the vertebrae. Events following birth might have similar results. Thus the form of a flexed tail is probably the resultant both of hereditary and environmental influences.

LINKAGE RELATIONS OF THE FLEXED GENE

Our observations indicate that there is no linkage between flexed and albinism, but data are not available for testing linkage with other char-

acters. The backcross generation was available for this study. Two types of backcrosses were made: first, the flexed albino male×the F_1 colored female (produced by the P_1 cross flexed albino male×normal tailed colored female), and second, the reciprocal of the above cross. The progeny of the cross flexed albino male×F_1 colored female were as follows:

Flexed albinos:	108	Flexed colored:	106
Intermediate or doubtful tailed, albinos:	9	Intermediate or doubtful tailed, colored:	9
Total counted as flexed albinos:	117	Total counted as flexed colored:	115
Normal tailed albinos:	172	Normal tailed colored:	177

The "intermediate or doubtful tailed" were, as has been mentioned, those cases where the presence of a very slight flexure or an indistinct stiffness in the tail justified the view that they could not be normals. We have assumed that such mice were extreme variants in the flexed distribution.

The analysis of the data is complicated by the differential death rates which seemed to be higher among the flexed than the normals in these mixed litters, and by such extreme variability of the flexed that some of them looked like normals. So for clarity it may be well to set down the genetic formulae.

F = gene for normal tail. f = gene for flexed tail.
C = gene for color. c = gene for albinism.

P_1 zygotes: *ffcc* male × *FFCC* female
P_1 gametes: *fc* *FC*
F_1 females: *fc*·*FC*
F_1 ova : *fc*+*FC*+*fC*+*Fc*

When such F_1 females are mated with flexed albino males, the backcross progeny should be,

ffcc (flexed albinos) + *FfCc* (normal tailed colored) + *ffCc* (flexed colored; crossovers) + *Ffcc* (normal tailed albinos; crossovers)

The deficiency of flexed animals in the backcross generation was, as has been repeatedly asserted, probably due to a higher death rate among the flexed, and to flexed individuals that looked like normals. The overlapping can not have been very extensive in this particular backcross population, because among the large undepleted litters, where all the postnatal and

part of the prenatal deaths had been eliminated, 48.3 percent of the young were flexed.

The crossover percentage may be computed in various ways. What percentage of the F_1 flexed bearing germ cells were of the crossover type (*fC*)? The death rate among the *ffcc* young was probably about the same as among the crossover class, *ffCc*. The percentage of flexed albino young (*ffcc*) which were overlaps (that is, seemed to be normal tailed albinos, (*Ffcc*) was probably about equal to the percentage of flexed colored young (*ffCc*) which likewise were overlaps (looking like the normal tailed colored, *FfCc*). Thus the agencies which decreased the *ffcc* class undoubtedly operated with the same intensity on the *ffCc* crossover group, so that the percentage of flexed backcross young which had colored hair was probably about the same as the percentage of flexed bearing F_1 germ cells which carried the color factor. Thus computed, the percentage of crossing over was 49.57 ± 2.21 percent, which does not differ significantly from the 50 percent to be expected if no linkage exists.

In similar fashion one may determine what percentage of the F_1 germ cells that carried the gene *F* for normal tail, also bore the factor for albinism *c*. This becomes a matter of determining what percentage of the normal tailed young were albinos (*Ffcc*). As just pointed out, each of the two normal tailed classes no doubt contained a few flexed overlaps, but it has also been shown that they were probably not numerous in either case. The crossover percentage for the *F* bearing ova would be: $\frac{172}{172+177} \times 100 = 49.28 \pm 1.81$ percent.

If we use all four classes of backcross young to compute the percentage of crossing over by the conventional method, we get 49.40 ± 1.40 percent.

A limited amount of data (83 animals) was secured from the reciprocal cross, F_1 male (produced by the P_1 cross flexed albino male $\times$ normal tailed colored female) $\times$ flexed albino female. All four classes were used to compute the crossover percentage, which was found to be 51.81 percent. Thus albinism and the flexed gene are not located on the same chromosome, which means, also, that flexed is not linked with the genes known to be linked with, or allelomorphic to, albinism (full color, chinchilla, extreme dilution, dark-eye, pink-eye, non-shaker, shaker).

Inspection of the data presented in the tables shows that the flexed character is not sex-linked.

SUMMARY

1. The flexed tailed mutation was discovered in a stock of normal mice. It is characterized by stiff angular bends or spirals, or by rigid segments without flexures.

2. The character is highly variable, some tails being extremely bent in one or more places, while a few homozygous flexed animals can be distinguished from normals only by breeding tests.

3. The progeny of flexed×flexed crosses are nearly all flexed, the few exceptions being animals whose tails are normal but which are probably all homozygous flexed. There is evidence that the flexed character may be suppressed by other factors.

4. Short tail is an hereditary variation, found in the flexed population, which is probably due to several genes.

5. The flexed tailed character is recessive.

6. The percentage of flexed young produced by the backcross (F_1 ×flexed) approaches 50 percent when the postnatal and part of the prenatal deaths are eliminated from the data.

7. The normal tailed female offspring of F_1×flexed matings have the same genetic constitution as F_1's.

8. The percentage of flexed animals in the F_2 generation is in agreement with the views that flexed is a recessive character which can be suppressed by modifying genes, and that the death rate for flexed mice is higher than for normals.

9. The coefficient of correlation between the grades of flexure in flexed parents and their offspring is $+.243 \pm .029$, indicating that the grade of flexure is to some extent inherited.

10. The flexed gene is not linked with albinism, and it is not sex-linked.

LITERATURE CITED

DE ABERLE, S. B., 1927 A study of the hereditary anaemia of mice. Amer. J. Anat. **40**: 219–249

BLANK, E., 1916–1917 Die Knickschwänze der Mäuse. Arch. EntwMech. Org. **42**: 333–406.

DANFORTH, C. H., 1930 Developmental anomalies in a special strain of mice. Amer. J. Anat. **45**: 275–287.

DOBROVOLSKAÏA-ZAVADSKAÏA, N., 1927a Sur la mortification spontanée de la queue chez la souris nouveau—née et sur l'existence d'un caractère (facteur) héréditaire "non viable." C. R. Soc. Biol. **97**: 114–116.

1927b Brachyurie, accompagnée de coudures et "structure génétique" de la queue chez la souris. Also, Une nouvelle mutation "bout flexible" de la queue chez la souris. C. R. Soc. Biol. **97**: 1583–1587.

1928a L'irradiation des testicules et l'hérédité chez la souris. Arch. Biol. Paris **38**: 457–501.

1928b Nouvelles mutations du type hélicoïde de la queue chez la souris. C. R. Soc. Biol. **98**: 1304–1306.

1928c Sur une souche de souris, présentant une mutabilité insolite de la queue. Académie des Sciences, October: 615–617.

1928d Contribution a l'étude de la structure génétique d'un organe. C. R. Soc. Biol. **99**: 1140–1143.

1929 The problem of species in view of the origin of some new forms in mice. Biol. Rev. **4**.

DOBROVOLSKAÏA-ZAVADSKAÏA, N. and KOBOZIEFF, N., 1927a Sur la reproduction des souris anoures. C. R. Soc. Biol. **97**: 116–118.

1927b Sur une nouvelle mutation de la queue, "queue filiforme," chez la souris. C. R. Soc. Biol. **97**: 1140–1142.

1928 Suspension du développement embryonnaire d'une vertèbre dans la queue de souris et les mutations qui en résultent. C. R. Soc. Biol. **98**: 1301–1303.

1930 "Cône-filament" et "pendeloque," deux mutations opposées de la queue chez la souris C. R. Soc. Biol. **104**: 969–971.

HUNT, H. R., 1932 The flexed tailed mouse. Proc. Sixth Int. Congress Genetics **2**: 91–93.

HUNT, H. R. and PERMAR, D., 1928 Flexed tail, a mutation in the house mouse. Anat. Rec. **41**: 117.

PLATE, L., 1910 In Festschrift zum Sechzigsten Geburtstag Richard Hertwigs. Jena: G. Fischer.

ANEMIA IN THE FLEXED TAILED MOUSE, MUS MUSCULUS

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HUNT, MIXTER, and PERMAR (1933) have described the mode of inheritance of flexed tail in the house mouse, a mutation discovered by HUNT in the rodent colony of the Department of Zoology at the MICHIGAN STATE COLLEGE. Flexed tailed mice are characterized by fusions between adjacent caudal vertebrae, and as a rule by angular or spiral bends in the tail. The character is a Mendelian recessive, but there is a deficiency of flexed individuals in F_2 and backcross populations because the mutants have a higher death rate than their normal siblings. Newborn flexed mice are anemic, and the purpose of this paper is to describe the inheritance and characteristics of this anemia.

To avoid the reprinting of tabular data from the first paper, the tables of the two publications are numbered as one series, tables 1 to 10 being in the first, and 11 to 17 in this contribution.

THE INHERITANCE OF ANEMIA

Early in the experiments the writer (H. R. HUNT) noticed that in litters bred by matings between flexed and F_1 animals, the flexed young were considerably lighter in color than their normal tailed siblings. The hairless condition of newborn mice gives them a reddish color, for the blood shows through the skin. The lighter pink of the flexed mouse immediately after birth is frequently more noticeable on some parts of the body than others. The hips and top of the head are particularly favorable places for detecting the paleness. It seemed to the writer that the lightness of the flexed young was probably due to an anemia of some kind, so Mr. RUSSELL MIXTER undertook a study of the numbers of erythrocytes and the hemoglobin contents of blood from flexed and normal tailed animals at different ages.

The anemic appearance of young flexed mice varies, in some animals the condition being very obvious, while in others careful scrutiny is necessary to detect any difference from the normal siblings. The litters should be examined for classification on the day of birth and by good daylight. Occasionally one can not decide definitely whether the young mouse is anemic or normal, and such cases have been designated as doubtful. Flexed young are usually anemic at birth, but straight tailed newborn mice frequently show this condition, and on the other hand a flexed ani-

mal is occasionally as red as its normal newborn siblings. These matters will be considered when discussing whether the flexed and anemic conditions are due to the same gene.

This section is intended to throw light on the mode of inheritance of anemia, which was discovered only after the work on the inheritance of flexed tail was under way. Thus the experiments were not designed for its study, so they did not definitely establish the way in which it is inherited. For example, we do not know whether this anemia breeds true, nor are we certain that our P_1 flexed animals were anemic at birth. However the writer's experiments accumulated considerable data all of which are consistent with the view that anemia, like flexed, is a Mendelian recessive, so the facts are presented as a contribution to the question and not as a final solution.

Table 11 summarizes the evidence concerning the inheritance of anemia. It includes the F_2 generation, the offspring of $F_1 \times$flexed matings, and the progeny of flexed males paired with normal females which, in turn, were bred by crosses between F_1 and flexed animals. Unfortunately no special effort was made to study anemia in the F_1 generation, but the records show that four litters containing 20 animals were observed, and that all were normal.

The F_2 generation will be considered first. Every doubtful case was excluded from the F_2 data presented in table 11. All the dead have been eliminated, for the identification of a dead individual as anemic or normal is hazardous. Also, if the classification of one or more animals in a litter was uncertain, the whole litter was excluded from the table. The small number of such doubtful cases may actually have been selected at random from the normal and anemic classes, but there is ground for the suspicion that they had mild cases of anemia. To have eliminated such cases without disposing of their normal siblings would have unjustifiably reduced the percentage of anemics. There were 1373 F_2's, of which 1076 were normal and 297 clearly anemic. Thus $21.63 \pm .75$ percent were anemic.

This is 3.37 percent less than the 25 percent expected on the assumption that anemia is recessive, yet it approximates 25 percent closely enough to arouse the suspicion that anemia is actually such a character. It has already been shown that flexed animals probably have a higher prenatal death rate than normals, so the deficiency in anemic young may be due to the same cause. This difficulty may be partly overcome by considering only large litters. In table 11 the data are given for litters of 7 or more, and 9 or more young. A population consisting of litters of nine or over on the day of birth must have been selected very strongly against a prenatal death rate, and therefore against any differential rate as between normals and anemics. This technique has been discussed already in con-

TABLE 11
The inheritance of anemia.

CLASSES OF NEWBORN MICE	FREQUENCY OF ANEMIA AMONG NEWBORN MICE								
	IN ALL LITTERS			IN LITTERS OF 7 OR MORE YOUNG			IN LITTERS OF 9 OR MORE YOUNG		
	NORMALS	ANEMICS	PERCENT ANEMIC	NORMALS	ANEMICS	PERCENT ANEMIC	NORMALS	ANEMICS	PERCENT ANEMIC
F_2 generation	1076	297	21.63± .75	755	216	22.25± .90	363	108	22.93±1.31
Progeny of F_1×flexed	490	424	46.39±1.11	360	322	47.21±1.29			
Progeny of normal females (bred by the cross F_1×flexed)×flexed males	241	195	44.72±1.61	105	89	45.88±2.41			

nection with the inheritance of flexed (HUNT, MIXTER, and PERMAR 1933). There were 971 young in litters of 7 or more per litter. Of these, 216 were anemic, or $22.25 \pm .90$ percent. Litters including dead animals were excluded, not only on account of the possibility of classifying the dead erroneously, but also because death may have occurred before birth, and the aim was to reduce the effect of the prenatal death rate as much as possible. Where the condition of the blood was doubtful in one or more animals, the whole litter was thrown out of the computation. If from the above litters of 7 or more young, those containing 9 and over are considered, there were 108 anemics and 363 normals, or 22.93 ± 1.31 percent of anemics. Partial correction for a prenatal mortality thus raises the percentage of flexed animals to within about 2 percent of the 25 percent that should be approximated if anemia is due to a single recessive factor. This justifies the suspicion that the type of anemia with which we are dealing may be caused by such a gene.

The fact should not be overlooked, however, that to secure even such a high percentage of anemic young as 22.93 percent in F_2, the prenatal death rate among the anemic young might be quite high. The following computations illustrate this point, assuming that anemia is a simple recessive character.

Assumed prenatal death rates of normals.	Computed prenatal death rates of anemics.
0 percent	10.7 percent
3 percent	13.6 percent
5 percent	15.0 percent

The prenatal death rates would have to be not far from 10 percent higher among anemics than normals, even in the litters of 9 or more, to give 22.93 percent of anemics. The anemia may, conceivably, so handicap the fetus that such a differential mortality actually exists. A conclusive study of prenatal death rates, using comparable normals as controls, is highly desirable.

Table 11 shows the results of backcrossing F_1's with flexed animals. Let us first consider all litters, regardless of their sizes. Here again all dead young were discarded in the computations, and in addition all litters were eliminated which contained one or more animals whose blood status was doubtful. The reasons for these omissions have already been discussed in connection with the F_2 generation. There were 914 young mice of which 46.39 ± 1.11 percent were anemic. This is 3.61 percent less than the 50 percent to be expected in a backcross generation if the character is a simple Mendelian recessive. The table gives the data, also, for litters of 7 or more. Litters containing dead young, or young which could not with certainty

be classified either as normal or anemic, have been omitted. This selection of litters on the basis of large size would be expected to increase the percentage of anemic animals if they have more than the normal prenatal mortality. 47.21 ± 1.29 percent were anemic, which was a small and statistically unreliable increase over 46.39 ± 1.11 percent. Again the facts, as far as they go, justify the idea that the anemia may be the expression of a recessive gene, for the percentages are suspiciously close to 50 percent.

The last horizontal column of table 11 deals with the results of crossing flexed males with normal females which were bred by crosses of F_1 with flexed animals. The litters used in computing the percentages were selected in exactly the same ways as in the two preceding experiments. If the anemia is due to the flexed gene itself, or to a recessive gene very closely linked with flexed, then one would expect an approximation to 50 percent among the offspring from such matings. The percentages of anemics actually found were 44.72 ± 1.61 percent for all the litters, and 45.88 ± 2.41 percent in litters of 7 or more. These percentages, again, are suggestive.

STUDY OF THE BLOOD OF THE FLEXED TAILED MOUSE

Mr. Russell Mixter was assigned the task of determining the numbers of erythrocytes and leucocytes in the bloods of flexed and normal mice at birth and subsequent ages, and of measuring the hemoglobin percentages in the same strains. These studies, together with the material on inbreeding already reported, were submitted as a graduate thesis in the Department of Zoology at the Michigan State College. No attempt will be made to review the voluminous literature on anemia in man and animals, but Mr. Mixter's findings will be presented together with some comments on the genetic significance of his observations.

It was necessary, of course, to compare the cell counts and hemoglobin percentages of flexed mice with corresponding data from normal controls to determine whether the flexed animals were actually anemic. Two types of controls were used: normals carrying flexed (heterozygotes), and homozygous normals. Flexed and heterozygous normal tailed young were secured by mating heterozygotes with flexed. Seven heterozygous (F_1) normal males were mated with 14 flexed females. In addition, 1 flexed male was bred with 6 heterozygous normal females. The mixed litters produced by these matings provided material for reliable comparisons between normal and flexed young. As a rule, when observations were made on a flexed animal the same procedure was carried out on a normal litter mate. Thus such factors as health of the mother, temperature of the animal house, chance fluctuations in the quality of the food, external and internal parasites, et cetera, which may have affected the erythrocyte and hemoglobin content of the flexed animals, would produce, presumably, similar effects

on the heterozygous controls, so that the differences between the groups were due in the main to the normal gene of the heterozygous controls.

The other type of control was the homozygous normal. These mice were produced by 1 homozygous normal male mated with 8 homozygous normal tailed females. The offspring of these crosses lived under approximately the same conditions as the flexed animals, so differences between them could not have been due, in any considerable degree, to the environment. But the heterozygous siblings of the flexed young were doubtless better controls than the unrelated homozygotes, because the former more than the latter must have resembled the flexed young in the distribution of genes other than the flexed gene. It is important to remember these distinctions, for, as will appear later, the two control series differed from one another as well as from the flexed mice.

Blood cell counts and hemoglobin determinations were made at birth, at one, two, three, and four weeks after birth, and further at six, eight, ten, and twelve weeks. Adults also were investigated. These were mainly the parents of the young on which observations were made. Four of the flexed adults were not parents of the mice used in the blood problem, but of those recorded in the inbreeding experiment. The ages of the adults ranged from six months to nearly two years.

The blood could not be secured by the same method throughout the whole series. Newborn animals are so small and have such a limited supply of blood that the sample was taken from the severed neck after decapitation. Thus no counts at later dates were ever made on animals counted at birth. Sometimes the blood of week-old young was secured in this manner, but more often it was from the femoral vein. Wherever possible a mouse yielded samples at the end of the first, second, third, et cetera, weeks. From two weeks on, blood was usually obtained from the severed tip of the tail, several millimeters being cut off each time a sample was secured. Occasionally the blood came from the leg if the tail would not bleed. The flexed and their normal controls were treated alike; if the sample was taken from the leg of one, it was secured from the same place on the other. The blood from the decapitated newborn mice was probably slightly diluted with lymph, but such dilution occurred both in the flexed and their normal controls, so that the method of securing the sample did not invalidate the comparison.

Mr. MIXTER used a Will certified pipette in securing samples when both erythrocytes and leucocytes were to be counted, and a Trenner pipette when the number of leucocytes only was to be determined. A 1 percent solution of sodium chloride tinged with a small quantity of Gentian Violet was used when both types of cells were counted, while a $1\frac{1}{2}$ percent acetic acid solution tinged with Gentian Violet was utilized for the white cell

counts alone. The blood was diluted 200 times with the salt solution and only 20 times with the acid solution. The first determinations were made with a Levy-Hauser single counting chamber, the average of two successive counts being taken as the record for the animal. The Levy-Hauser double counting chamber was used in most cases, for two samples could then be obtained at one filling of the chamber. The percentage of hemoglobin was estimated by using a Tallquist color scale, which is adequate for an approximate, but not for a highly accurate determination. This scale gives the concentration (percentage) of hemoglobin in a blood sample as compared with normal human blood whose hemoglobin content is taken as 100 percent.

It should be understood clearly that Mr. MIXTER'S object was to determine the cause of the pale color in most of the newborn flexed mice. It might, conceivably, have been due to an abnormally thick skin. He selected animals for the study of the blood because they were flexed tailed and not on the basis of color at birth. Consequently this is a report on the blood of flexed tailed mice and not an extensive investigation of anemia. The genetic relation of flexed tail to anemia, the histology of this type of blood, and the fetal history of anemia are problems yet to be attacked.

The erythrocytes of the flexed animals and the two types of controls will be considered first, after which the hemoglobin and leucocyte content will be discussed. Table 12 gives the average numbers of red cells per cubic millimeter of blood in the flexed and normal mice, beginning at birth, then at weekly and fortnightly intervals up to twelve weeks, and finally at the adult stage. The number of individuals used in making each computation is indicated. The data of table 12 are plotted in figure 1. Table 13 gives the frequency distribution of red blood cells at birth in the three categories of animals. The purpose of the table is to show the extent to which the flexed and their controls overlap.

Table 12 and figure 1 reveal that there was a rapid increase of erythrocytes from birth to about the fourth week in the controls and the flexed. For some reason the red cells of the homozygous normals remained nearly constant from the second to the third week, but the fourth week brought an augmentation which compensated for this lag. After about the fourth week there was a slower growth in erythrocyte content until the eighth or tenth week, when the adult level was reached. The erythrocytes of the flexed mice increased from 3,550,000 per cubic millimeter at birth to 11,360,000 at eight weeks; this latter number remained about the same at ten and twelve weeks, and at the adult stage. The heterozygous normals started at birth with a larger number of red cells (4,850,000 per cubic millimeter) than the flexed, the number increasing relatively rapidly to the fourth week, but not so rapidly as in the flexed animals, as is shown by the

TABLE 12

Red blood cells of flexed and normal mice.

AGE OF THE MICE	FLEXED MICE		HETEROZYGOUS NORMAL MICE		HOMOZYGOUS NORMAL MICE	
	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED
Birth	3,550,000	27	4,850,000	22	4,020,000	15
1 week	5,130,000	26	5,450,000	31	5,090,000	15
2 weeks	6,810,000	23	6,840,000	22	6,600,000	15
3 weeks	8,120,000	20	7,710,000	20	6,750,000	15
4 weeks	10,050,000	17	9,510,000	17	9,200,000	12
6 weeks	10,510,000	15	10,090,000	15	9,870,000	4
8 weeks	11,360,000	13	10,320,000	13	10,000,000	2
10 weeks	11,170,000	15	10,690,000	14		
12 weeks	11,500,000	14	10,790,000	14		
Adults	11,210,000	16	11,040,000	15	9,690,000	12

fact that the normal curve crossed the flexed at the second week. Thereafter the heterozygous normals had a lower concentration of erythrocytes than the flexed. These normals reached a level at about the tenth week which was but little lower than for the twelfth and the adult stage. The homozygous normal controls showed a birth count of 4,020,000 red cells, which was intermediate between the flexed and heterozygotes, but closer to the former than the latter. The homozygous normal and the flexed curves intersected at one week, and thereafter the homozygotes had fewer erythrocytes than either the heterozygotes or the flexed.

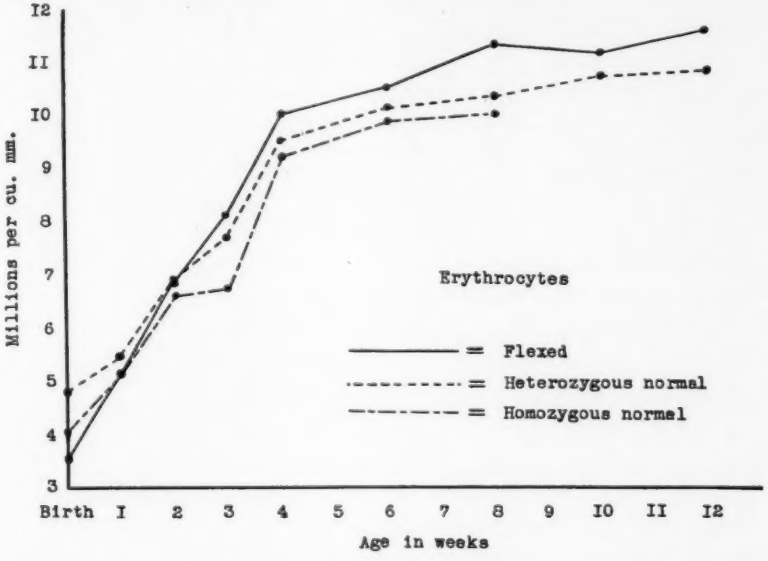

FIGURE 1.

The lack of erythrocytes which on the average afflicts the flexed animals is characteristic of early infancy, the group as a whole rapidly recovering from the disability. Let us examine critically the evidence for this statement. Table 13 shows the frequency distribution of red cell counts in the flexed and controls at birth. There was much overlapping among the three types, nearly all the homozygous normals falling within the range of variation of the flexed. The probable causes of these overlaps will be discussed later. We are not concerned for the present with the blood counts after the second week, though the apparent superiority of the flexed animals after this age may be worth further study. The average numbers of erythrocytes at birth were as follows:

Flexed: 3,550,000 ± 99,900
Homozygous normals: 4,020,000 ± 112,700
Heterozygous normals: 4,850,000 ± 111,400

TABLE 13

Frequency distribution of red blood cells in flexed and normal mice at birth.

CLASSES (NUMBER OF RED CELLS PER CUBIC MILLIMETER)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
2,400,000–2,490,000	2	..	..
2,500,000–2,590,000	..	..	..
2,600,000–2,690,000	4	..	..
2,700,000–2,790,000	..	..	..
2,800,000–2,890,000	2	..	..
2,900,000–2,990,000	1	..	1
3,000,000–3,090,000	1	..	1
3,100,000–3,190,000	1	..	..
3,200,000–3,290,000	1	..	1
3,300,000–3,390,000	2	..	..
3,400,000–3,490,000	..	..	..
3,500,000–3,590,000	..	1	1
3,600,000–3,690,000	..	..	2
3,700,000–3,790,000	..	..	..
3,800,000–3,890,000	3	..	..
3,900,000–3,990,000	1	2	1
4,000,000–4,090,000	1	2	2
4,100,000–4,190,000	..	1	..
4,200,000–4,290,000	1	1	..
4,300,000–4,390,000	3	1	2
4,400,000–4,490,000	..	..	..
4,500,000–4,590,000	1	1	1
4,600,000–4,690,000	1	2	..
4,700,000–4,790,000	1	..	..
4,800,000–4,890,000	1	..	..
4,900,000–4,990,000	..	..	3
5,000,000–5,090,000	..	2	..
5,100,000–5,190,000	..	2	..
5,200,000–5,290,000	..	1	..
5,300,000–5,390,000	..	1	..
5,400,000–5,490,000	..	1	..
5,500,000–5,590,000	..	..	..
5,600,000–5,690,000	..	..	..
5,700,000–5,790,000	..	1	..
5,800,000–5,890,000	..	..	..
5,900,000–5,990,000	..	1	..
6,000,000–6,090,000	..	1	..
6,100,000–6,190,000	..	..	..
6,200,000–6,290,000	..	..	..
6,300,000–6,390,000	..	..	..
6,400,000–6,490,000	..	..	..
6,500,000–6,590,000	..	1	..

The difference between the flexed and the homozygous normals was 470,000 ± 151,000 erythrocytes per cubic millimeter. This difference was only 3.1 times as large as its probable error, so it was just barely significant statistically according to conventional standards. The difference between the flexed and the heterozygotes was 1,300,000 ± 150,000, and since this difference was 8.7 times its probable error it can scarcely have been due to chance. Thus the flexed animals are definitely deficient in erythrocytes at birth.

The evaluation of these differences should take into account the nature of the controls. The heterozygous normals were, theoretically, better controls than the homozygotes. This was particularly true of the newborn young. Usually the blood count of a flexed animal was paired with the count for a normal litter mate. Thus a flexed animal at birth had been subjected, as a rule, to about the same prenatal environmental influences as the normal heterozygous mouse which was selected to serve as its control, for both had developed in the same uterus at the same time. The homozygous controls, of course, were always developed in uteri which were carrying no flexed mice. Their uterine environment may have been quite different, on the whole, from that of the flexed animals with which they were compared within a few hours after parturition. Also, the heterozygous normal controls were probably, on the whole, genetically more like the flexed animals than were the homozygotes, for the heterozygotes came from the same group of parents as the flexed. So if there are factors other than the one with which we are now concerned that affect the number of erythrocytes and the amount of hemoglobin, the heterozygotes would be expected to resemble the flexed in the possession of these factors more closely than the homozygotes.

Thus it is conceivable, though of course not proved, that some of the homozygous normal controls may have possessed factors, other than the main factor for anemia, which depressed the erythrocyte content at birth, and that such factors were less frequent in the flexed animals and the closely related heterozygous controls. This might account for the relatively small difference between the flexed and homozygotes at birth. Such an explanation of the higher counts in the heterozygous normals at birth in contrast with the homozygotes and the flexed is, I think, more plausible than an assumption to the effect that heterozygosity with respect to the flexed gene raises the erythrocyte count over the normal level in some mysterious manner.

To conclude, flexed animals as a group are lacking in red blood cells at birth, but on the average they reach the normal level when one to two weeks old. This anemia is not a permanent or fatal defect.

The facts with regard to hemoglobin are presented in tables 14, 15, and

TABLE 14

Hemoglobin of flexed and normal mice.

AGE OF MICE	FLEXED MICE		HETEROZYGOUS NORMAL MICE		HOMOZYGOUS NORMAL MICE	
	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED
Birth	38.2	44	60.3	41	56.9	26
1 week	49.0	43	57.9	46	53.3	15
2 weeks	54.0	36	56.3	31	56.0	15
3 weeks	58.2	36	57.0	33	52.0	15
4 weeks	58.2	31	61.5	30	60.8	12
6 weeks	60.7	15	61.7	15	61.3	4
8 weeks	65.4	13	66.5	13	60.0	2
10 weeks	65.0	15	64.3	14	....	..
12 weeks	67.1	14	67.5	14	....	..
Adults	68.8	16	64.3	15	65.8	12

TABLE 15

Frequency distribution of hemoglobin in flexed and normal mice at birth.

CLASSES (PERCENTAGE HEMOGLOBIN)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
30	13	..	..
35	7	..	..
40	10	2	..
45	12	..	2
50	1	1	2
55	1	3	8
60	..	25	13
65	..	6	..
70	..	3	1
75	..	..	..
80	..	1	..

TABLE 16

Frequency distribution of hemoglobin in flexed and normal mice at one week of age.

CLASSES (PERCENTAGE HEMOGLOBIN)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
35	1	..	..
40	5	..	..
45	14	..	..
50	11	9	7
55	6	11	6
60	6	19	2
65	..	6	..
70	..	..	..
75	..	..	..
80	..	1	..

16, and in the curves of figure 2. As previously mentioned, the Tallquist method was used in determining the relative concentrations of hemoglobin. Table 15 shows the frequency distribution for the flexed and normal mice at birth, while table 16 gives such data at the age of one week. Hemoglobin is the substance which gives the blood its characteristic red color, so if the paleness of the infant mice is due to an anemia, one would expect to find less hemoglobin in the blood of the newborn flexed than in the two groups of controls. Such was the case.

The flexed animals had at birth an average hemoglobin concentration of 38.2 percent of the normal human standard. This increased rapidly during the first three weeks, reaching the level of the controls during the third

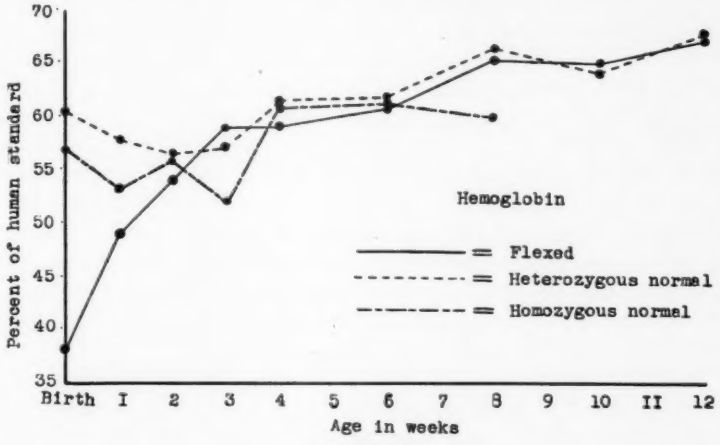

FIGURE 2.

week, thereafter slowly increasing until an adult percentage of 68.8 percent was attained. The heterozygous normal mice began postnatal life with an average hemoglobin percentage of 60.3 .This decreased slightly during the first and second weeks, then came a gradual but irregular rise which carried the hemoglobin percentage to 64.3 percent in the adult. The curve for these heterozygous controls followed closely the curve for the flexed from the third to the twelfth week, though the flexed adults had 4.5 percent more hemoglobin than the adult controls. The flexed animals increased their hemoglobin by over 30 percent from birth to adulthood, while the corresponding increase for the heterozygous normals was only 4 percent. The homozygous normals had 56.9 percent of hemoglobin at birth; the percentage declined irregularly until the third week, after which came a rise which carried the hemoglobin content to 65.8 percent in the adult. No observations were made at the end of the tenth and twelfth weeks. The

more irregular course of the curve for the homozygous controls may have been due to the relatively small numbers of individuals observed. The hemoglobin content of the homozygotes at birth was, as with the erythrocyte counts, intermediate between the flexed and the heterozygous controls. It is worthy of note, also, that though the flexed mice enter the world markedly deficient in hemoglobin, on the average, yet the survivors not only speedily overtake the normals when about two weeks old, but on the whole slightly exceed them at maturity. Thus the anemia from which the flexed mutants suffer automatically disappears during infancy, in this respect differing decidedly from the severe lethal anemia found by DE ABERLE in homozygous dominant white animals.

Table 15 shows that there was some overlapping in the distributions of flexed and control groups at birth, but this was not as pronounced as with the distributions of erythrocyte counts presented in table 13, which shows that the homozygous normals fell almost completely within the range of variation of the flexed, and that half the heterozygous normals did the same. In table 15 only 15 percent of the heterozygous and 46 percent of the homozygous normals come within the limits of the flexed distribution. Moreover, the differences between the flexed animals and the controls at birth were statistically significant, as the following computations show.

Average percentage of hemoglobin for the flexed: $38.2 \pm .68$ percent
Average percentage of hemoglobin for the homozygous normal controls: $56.9 \pm .69$ percent
Average percentage of hemoglobin for the heterozygous normal controls: $60.3 \pm .70$ percent

The difference between the flexed and homozygous normal controls was $18.7 \pm .97$ percent, and since this difference was 19.3 times as large as its probable error it was highly significant statistically. The percentage for the heterozygous straights exceeded that for the flexed by $22.1 \pm .98$ percent. This difference was likewise of great significance because it was 22.6 times the size of its probable error. Thus the hemoglobin percentages emphatically testify that, on the average, the flexed group was anemic when born.

The flexed and anemic animals were more nearly alike at one week of age than at birth, but the differences were still significant, as the following data show.

Average percentage of hemoglobin for the flexed: $49.0 \pm .66$ percent
Average percentage of hemoglobin for the homozygous normal controls: $53.3 \pm .61$ percent
Average percentage of hemoglobin for the heterozygous normal controls: $57.9 \pm .57$ percent

The percentage of hemoglobin in the heterozygous controls was $8.9 \pm .87$ percent higher than in the flexed animals. This difference was over ten

times the magnitude of its probable error. The homozygous normals had $4.3 \pm .90$ percent more hemoglobin than the flexed, and this difference too was significant because it was 4.8 times as large as its probable error.

Thus the flexed animals attain the hemoglobin level of the normals at about the second week, while the number of erythrocytes reaches the normal level perhaps a little earlier (fig. 1), but all the data show that the flexed animals are on the average deficient in erythrocytes and hemoglobin at birth, but that these defects are rapidly remedied.

An examination of figures 1 and 2 shows at a glance that the individual erythrocytes of both types of controls were presumably, on the average, more richly endowed with hemoglobin at the time of the mouse's birth than were the erythrocytes of the flexed individuals. The red cell curves of all three groups follow much the same course, a rapid infantile increase being followed by a lower rate. This was not the case with the hemoglobin percentages. The hemoglobin and erythrocytic curves of the flexed animals resemble one another, but the hemoglobin percentages of the two groups of controls did not show the marked infantile increase which was found in the flexed group. Whereas the flexed animals were 30.6 percent below their adult level, the controls had only 4.0 percent and 8.9 percent less hemoglobin at birth than when full grown.

Quantitative estimates of the relative average amounts of hemoglobin in each erythrocyte of the control types and flexed at birth, and of the adults will be of interest. However, the reader is cautioned not to take these estimates too seriously, for the numbers of mice used were not large; also, as will be pointed out shortly, the controls may possibly have included a few anemic animals and the flexed group a number of normal blooded individuals.

Suppose we define a unit of hemoglobin as the average amount carried by an erythrocyte of a flexed animal at birth. Suppose, further, that we use throughout the computations the volume of blood which in a newborn flexed mouse contains 100 erythrocytes. We may now find, by using the data in tables 12 and 14, how many units of hemoglobin, on the average, an erythrocyte of a flexed animal carries when adult. The hemoglobin of flexed mice increased from birth to adulthood by 80.1 percent of the amount present at birth. Thus there would be 180.1 units of hemoglobin (100+80.1) in the volume of adult blood used as our standard volume. The number of erythrocytes, during this period, increased by 215.8 percent of the number present at birth, so that there would be 315.8 erythrocytes per unit volume of blood in the adult (100+215.8). The average hemoglobin quota of the adult's erythrocytes in flexed mice would therefore be, 180.1 units ÷ 315.8 erythrocytes = .57 units. Thus the hemoglobin load of each erythrocyte decreased from 1.00 unit at birth to .57.

The hemoglobin per red cell at birth in the heterozygous normal controls is computed as follows. These controls had 1.579 as much hemoglobin per unit volume of blood at birth as did the flexed (60.3 percent, hemoglobin at birth of the controls ÷ 38.2 percent, hemoglobin at birth of the flexed). The heterozygous controls had also 1.366 as many erythrocytes per unit volume of blood at birth as the flexed mice (4,850,000, erythrocytes at birth of the controls ÷ 3,550,000, erythrocytes at birth of the flexed). The number of units of hemoglobin per erythrocyte for the heterozygous normals when born would be, therefore, $1.579 \times 100 \div 1.366 \times 100 = 1{:}16$ units.

To determine, next, the units of hemoglobin per red blood cell of the adult heterozygous controls, we make use of the facts that the hemoglobin of these increased over the content at birth by 6.6 percent of the amount at birth, and that the increment for the erythrocytes was 127.6 percent of the number at parturition. Thus the average hemoglobin quota in the red blood cell of the adults was $157.9 + (157.9 \times .066) \div 136.6 + (136.6 \times 1.276) = .54$ units.

The following summary brings together all the estimates from the available data.

Units of hemoglobin per erythrocyte for

	Flexed	Heterozygous normal controls	Homozygous normal controls
At birth	1.00	1.16	1.32
In adults	.57	.54	.63

The adult erythrocytes in all three types of animals are thus seen to have carried about the same quantities of hemoglobin, the flexed being intermediate between the controls. On the other hand, the controls' red blood cells were endowed at birth with from 16 percent to 32 percent more hemoglobin per cell than the flexed animals. Thus the blood of the newborn mutants was not only deficient in total hemoglobin content, and in erythrocytes, but each erythrocyte carried less than the normal quantity of hemoglobin. These estimates are probably of slight quantitative value, yet they seem to bring out another important fact about our flexed mutation.

Mr. MIXTER studied the leucocyte content also of his animals. His findings are summarized in table 17, which gives the average numbers of leucocytes per cubic millimeter at the different ages indicated from birth to maturity. All three types showed an irregular increase with advancing age, but the flexed animals did not differ significantly from the controls. The blood deficiencies of the flexed did not therefore involve the white cell content as a whole. Mr. MIXTER did not enumerate separately the different types of leucocytes, however, so it is conceivable that a deficiency of one type of leucocyte in the flexed may have been offset by an increase in another type.

TABLE 17

Average numbers of leucocytes per cubic millimeter.

AGE	FLEXED		HETEROZYGOUS NORMAL		HOMOZYGOUS NORMAL	
	NUMBER OF CELLS	NUMBER OF INDIVIDUALS	NUMBER OF CELLS	NUMBER OF INDIVIDUALS	NUMBER OF CELLS	NUMBER OF INDIVIDUALS
Birth	4297± 202	16	4129± 238	16	3993± 229	26
1 week	4192± 139	15	4481± 201	15	5000± 326	14
2 weeks	5086± 354	15	6167± 586	11	5000± 360	15
3 weeks	5805± 385	15	6502± 323	15	5600± 547	17
4 weeks	7435± 434	12	5340± 477	12	5600± 657	12
6 weeks	7700± 486	8	8000± 759	8	4500± 370	4
8 weeks	11200±1255	9	8700± 595	10	.. ..	..
10 weeks	8700± 690	12	10600±1106	11	.. ..	..
12 weeks	12700± 846	12	11800± 668	12	.. ..	..
Adult	10000± 834	15	9600± 748	13	11000± 738	12

ARE THE FLEXED AND ANEMIC CHARACTERS DUE TO THE SAME GENE?

We may now inquire whether the characters flexure and anemia are the results of a single recessive gene or of two recessive genes which are closely linked. This question can not be answered from the data at hand, but there are certain suggestive facts. If the flexed gene causes the anemia, then anemia and flexed should always occur together (unless some agencies, genetic or environmental, can suppress one of these characters without eliminating the other), and a normal tail would always be associated with the absence of this type of anemia. In other words there would be no cross-overs. But there is enough evidence at hand to shake one's faith in such a theory. Individuals that may possibly be the results of crossing over between genes for anemia and flexed were found.

Tables 2 and 4 contain such cases (see HUNT, MIXTER, and PERMAR, 1933). Among the 963 newborn backcross progeny reported in table 2, 78 are recorded as anemic but normal tailed. There are 1478 newborn F_2 mice in table 4, and of these 81 were normal tailed and anemic. Such a large number of exceptional cases would be good evidence that crossing over between genes for flexed and anemia had occurred if the classification of the newborn young could be depended on, but it can not. The identification of flexures in the tails of very young mice is sometimes difficult or impossible. Flexed is a highly variable character, as has been shown already. It grades into normality so perfectly that no absolutely defined boundary can be designated. Mr. MIXTER reports that among 415 offspring from flexed×flexed crosses, 3 anemic young were observed which at the age of 21 days showed no caudal stiffness or flexure. If such conditions are encountered in weaned flexed mice, it is obvious that the

absence of a bend in the very pliable tail of a newborn mouse does not prove it to be normal tailed.

The uncertainty in classifying the tails at birth is brought out by a study of the following 100 F_2 young which were branded with a hot needle on the day of birth, then observed when they reached the age at which the animals were usually given their final rating.

Normally red at birth, normal tailed at birth, and normal tailed at the final count:	75
Normally red at birth, doubtfully normal tailed at birth, and normal tailed at the final count:	12
Normally red at birth, straight tailed at birth, and doubtfully normal tailed at the final count:	1
Probably anemic, straight tailed at birth, and normal tailed at the final count:	1
Doubtfully anemic, straight tailed at birth, and doubtfully flexed at the final count:	1
Anemic, flexed at birth, and flexed tailed at the final count:	5
Anemic, doubtfully straight tailed at birth, and flexed tailed at the final count:	2
Anemic, doubtfully flexed at birth, and flexed tailed at the final count:	2
Anemic, straight tailed at birth, and the tail form transitional at the final count:	1

It will be seen that in five cases the tail was described as straight or doubtfully straight at birth, and that this verdict was reversed or rendered doubtful when the final count was made. Thus at least some of my newborn mice which were described as straight tailed anemics at birth may actually have been flexed, so the evidence from such cases does not prove that crossovers have occurred between genes for flexure and anemia.

Tables 2 and 4 record 5 flexed tailed newborn individuals (3 of them backcross young and 2 F_2's) which appeared to show the normal redness of non-anemic animals. Two of these turned out to be normal tailed, for no deaths occurred in the litters to which they belonged, and at the final counts after weaning, all the animals in their litters were found to have normal tails. This left three cases of supposedly flexed young which seemed normal blooded when the newborn were examined. In view of the fact that an ordinary muscular bend in the tail of an infant mouse might occasionally be taken for a flexure, no conclusions can be drawn from these three cases.

The writer found two newborn mice in a litter, not elsewhere recorded in this paper, both of which had flexed tails and a normal red color. Mr. MIXTER'S examination of the bloods showed one to have 5,890,000 red cells per cubic millimeter with 55 percent hemoglobin, and the other 65 percent hemoglobin and 5,300,000 red cells. Reference to tables 13 and 15 shows that the first animal had a normal erythrocyte count and a hemoglobin percentage which was at the extreme upper limit of the flexed distribution, while the second one was normal as regards hemoglobin percentage and red cell content, being entirely outside the range of flexed animals in both respects. To sum up the matter, the data are too scant and in many respects too uncertain to demonstrate whether or not the flexed and anemic traits are due to separate genes.

If recovery from anemia begins before birth, an occasional newborn flexed animal might have normal blood.

It should be kept in mind that Mr. MIXTER studied the blood of flexed animals rather than of anemics, so that his quantitative findings are not accurately descriptive of anemia. When newborn young were selected for blood counts and hemoglobin determinations, they were classified as flexed or straight tailed. Reference to tables 2 and 4 will show that there were quite a number of "intermediate or doubtful" cases where the writer was unable to classify the tails in newborn young. Considerable experience is required for this work. The tail may be bent as a consequence of muscular contraction on one side, or it may have a slight flexure which can be identified only after a rather close scrutiny. One of the main characteristics which the writer has assumed to identify newborn flexed tailed animals is a rather distinct angularity in the region of the flexion, usually quite different in appearance from the curvilinear aspect of a normal tail whose muscles are bending it. But there are undoubtedly cases where even experienced observers would not agree on the classification at birth. That is why it is so important to defer, as we have, final judgment as to the number of flexed animals in a litter until it is three to four weeks old, when the flexed condition can usually be identified positively.

Thus it is conceivable that Mr. MIXTER may have classified as flexed some newborn mice that were actually normal tailed. If the same gene causes both the flexed and anemic characters, such classification would place some normal blooded animals in the flexed category, thus causing the distributions of erythrocytes and hemoglobin percentages for flexed animals to overlap the ranges of both controls. Tables 13 and 15 show that such overlappings occur. Likewise, he may have classified as normal some animals that were homozygous flexed. This could happen wherever the genetically flexed animal would have shown only stiffness when full grown. Such events would extend the distributions of blood counts and hemoglo-

bin percentages of the heterozygous normal controls over into the ranges of the flexed animals. The above theory might account for the overlapping of the flexed and normal distributions of hemoglobin percentages, but it is difficult to believe that it explains the overlaps in the erythrocyte counts for flexed and homozygous normals, because their distributions cover almost the same range. Further study of the blood of newborn mice is needed to establish accurately the relationship between blood content and the paleness or redness of the mouse at birth.

MORTALITY OF FLEXED ANIMALS

One of the by products of Mr. MIXTER'S study of inbreeding was considerable information about deaths among infants in the second generation of inbred flexed animals. Unfortunately, normal controls bred and reared at the same time and under strictly comparable environmental conditions were not available, so that positive conclusions can not be drawn. The facts will be given, however. The record of deaths is as follows:

TOTAL NUMBER OF 2ND GENERATION MICE AT BIRTH (ONE ESCAPED)	NUMBERS DYING AT					TOTAL DYING	PERCENTAGE DYING
	1-3 DAYS	4-7 DAYS	8-14 DAYS	15-21 DAYS	22-28 DAYS		
625	79	60	32	14	17	202	32.3

About one-third of these flexed infant mice died before they were 28 days old. This seems like an exceedingly high mortality, presumably higher than the normal rate. The deaths were most numerous during the first three days, then sharply declined. It is worth noting that 84.7 percent of these deaths occurred during the first two weeks, the period of anemia, suggesting that this may have been an important contributory cause. Whatever the reasons are for this high mortality, it appears probable that young flexed mice are lacking in vitality, and this view is consistent with the fact that the writer has had some difficulty in maintaining the flexed stock.

The flexed mutation presents some interesting similarities to, and contrasts with, the hereditary anemia described by DE ABERLE (1927) in homozygous dominant white mice. DE ABERLE'S animals weighed less than normals, as did ours also (Mr. ALEXANDER A. ANDREWS' unpublished data on flexed), and both were much lighter in color than newborn normal mice. The anemia in the homozygous dominant whites was very severe, for all the afflicted young died within ten days after birth. Our flexed animals probably have a higher death rate than normals during infancy, but they recover from the anemia while still young. We can not accurately compare the blood of DE ABERLE'S and our anemics because, as has been

said already, the blood of flexed, rather than of anemic, mice was studied by Mr. MIXTER. DE ABERLE's newborn normal mice had an average of 4,740,020 ± 117,932 red cells per cubic millimeter, while the newborn anemics' average was 663,009 ± 15,714. Her normal controls had 89.78 ± 1.59 percent of hemoglobin, as judged by human standards, while the percentage for the anemics was 22.13 ± .56 percent.

SUMMARY

1. The anemia in the flexed tailed strain of mice is probably a recessive character.

2. Flexed animals are on the average deficient at birth both in hemoglobin and erythrocytes, but this defect disappears, on the whole, at about the age of two weeks.

LITERATURE CITED

DE ABERLE, S. B., 1927 A study of the hereditary anemia of mice. Amer. J. Anat. **40**: 219–249.

HUNT, H. R., MIXTER, R. and PERMAR, D., 1933 Flexed tail in the mouse, *Mus musculus* Cenetics **18**: 355–366.

CROSSING OVER AND GENE REARRANGEMENT IN FLOWERING PLANTS[1]

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HYPOTHESES OF CROSSING OVER

Kinds of gene rearrangement

"Crossing over" exists apparently in all those flowering plants which have been sufficiently investigated with regard to it. (By itself, it does not rearrange different genes, but only exchanges alleles.) Genetically it consists in exchange of normally equal and homologous segments of a gene string (MORGAN 1911, STURTEVANT 1915, MULLER 1916). It has been proved, by the microscope, to consist in the exchange, at meiosis, of normally equal portions of homologous chromonemas (CREIGHTON and MCCLINTOCK 1931, STERN 1931). It may thus be termed "homologous exchange". Since the process is a normal one, the mechanism is apparently so perfect that no genes are lost in the exchange.

"Reversed crossing over" consists in the exchange of different arms of homologous chromosomes, apparently at the fusal (spindle) constriction. It has been proved to occur at the origin of the secondary mutants of Datura, usually in trisomics, and probably at meiosis.

"Reciprocal translocation" (heterologous interchange) is much rarer than crossing over. It consists in the mutual substitution of usually unequal segments of heterologous chromosomes. It has been proved to occur cytologically as well as genetically (see BELLING 1927, STURTEVANT and DOBZHANSKY 1930, DOBZHANSKY and STURTEVANT 1931, MCCLINTOCK 1930). It seems to originate at both mitosis and meiosis. It may be caused by X-rays. Apparently genes may sometimes be lost in the process.

"Translocation" shows a segment of one chromosome terminally attached, usually to a heterologous chromosome (see especially PAINTER and MULLER 1929).

"Inversion" consists in a portion of the gene string or chromonema being turned round, end for end; genes being apparently sometimes lost in the process (see especially STURTEVANT 1931, and MCCLINTOCK 1931).

"Deletion" removes a non-terminal portion of the gene string, the gap closing up (see especially PAINTER and MULLER 1929). The portion removed may form a ring (MCCLINTOCK 1932).

[1] This paper gives the results of cytological work done under the auspices of the CARNEGIE INSTITUTION OF WASHINGTON. It is published posthumously and out of the order of receipt at the expense of the institution.

"Insertion" causes a segment of one chromonema to be placed within another gene string. Genes may be lost in the process. It seems quite rare.

"Deficiency" is usually the loss of a (terminal) segment of the gene string (see McCLINTOCK 1931). It originates at meiosis or mitosis.

The hypotheses

A successful hypothesis is normally based on *verae causae* (ascertained facts), while unsuccessful hypotheses are often marked by containing one or more *fictae causae* (imagined statements). The first hypothesis as to crossing over seems to have been that of JANSSENS (1909), who predicted exchange of parts of homologous chromosomes, from his observations of chiasmas. In this hypothesis he introduced the *fictae causae* of two strands first breaking accurately at corresponding points, and then these same two strands joining in an alternate way. These processes took place conjecturally at an overlap (or at a half twist). This "break and join" seems to the writer improbable mechanically. There would be needed an accuracy of position, in the two strands, of less than half a micron, with nothing to mark the breakage points. Without such accuracy, the crossing over would be unequal; which it is not (with one exception). Also the conditions required for a break are the reverse of those for a join.

A second hypothesis, stated in full by BATESON in 1911, demanded selective division of cells (somatic and gonial), in accurate ratios, precisely separating the pairs of alleles, and properly multiplying them, so as to result in numbers corresponding to the mathematical expression of certain combinations. This would seem to require unknown forces to bring it about, and was also contrary to the known relevant facts. It has been proved to be wrong.

A third composite hypothesis for crossing over required openings-out at diplotene, alternately at the primary split and the secondary split; these alternations being the *cause* of chiasmas. (It is possible that such alternations may occur as a *consequence* of the opening out of chiasmas at diplotene, in plants like Datura.) It also adopted the "break and join" of JANSSENS, the weak point of his hypothesis (SAX 1932). This alternate-opening hypothesis is negatived for certain liliaceous plants (and for Dendrocoelum) by the observed facts: that chiasmas are present at pachytene; that it is recognizably the primary split which opens out at early diplotene (a stage which has rarely been well figured); while the secondary split at this stage is responsible only for a two-lobed state of the chromomeres (GELEI 1921, BELLING 1931a). The sequence of these splits is especially clear in Lilium and Fritillaria, where the primary split can be followed from early pachytene to mid diplotene. This third hypothesis

thus contains three imaginary causes: alternate openings-out as a cause, not a consequence, of chiasmas; accurate double breaks; and special double joinings. Chromonemas have not been observed to break and join again at the chiasmas, as required by this hypothesis.

A fourth hypothesis, that of the present writer, attempts to postulate *verae causae*. It was framed after a study of the chromomeres and chromioles; and this was not the case with the three other hypotheses, which ignored the chromomeres. It also accounts for gene rearrangements, which the other hypotheses ignore. The chief supports of this fourth hypothesis are the following. (a) The absence of longitudinal division in the chromonema at leptotene; as observed especially by the writer (1931a), WENRICH (1917), GELEI (1921), and BELAR (1929a). (b) The proof of the complete equivalence of the two chromioles resulting from the secondary split in each chromomere (MENDEL 1866, SUTTON 1903, GELEI 1921). (c) The fact that only homologous chromomeres, including alleles, are connected transversely (that is, synapse) at zygotene; as observed by GELEI (1921), by the writer (1928, 1931a), and also by MCCLINTOCK (1931) in cases of inversion. (d) The fact that non-homologous chromomeres are connected longitudinally to their nearest neighbors by one, and only one, fiber; as observed especially by GELEI (1921) and by the writer (1928, 1931a). (e) That the *two* chromioles formed from each original chromomere by the secondary split are seen to have acquired a new fiber connection longitudinally (in addition to the old fiber), which joins *one* of them to the nearest non-homologous chromiole each way (GELEI 1921, BELLING 1928, 1931a, 1931b). (f) That such new connections are formed when the chromonema is dividing longitudinally (BELLING 1931a, 1931b). (g) That crossing over (exchange), inversion, translocation, interchange, and deletion can take place when the chromonemas are dividing longitudinally (BELLING 1931b).

PRELIMINARY KARYOLOGIC EVIDENCE

Chromonemas are normally unsplit at leptotene and during the resting stage

The writer has made a special investigation of this point, by means of smear preparations in iron-aceto-carmine, and also by smear preparations fixed with chromic-acetic-formalin and stained with iron-brazilin. The plants mostly used were *Tradescantia virginiana*, *Rhoeo discolor*, *Allium triquetrum*, *Lilium regale*, *Scilla sibirica*, and *Aloe striata*. The work resulted in the conclusion that the so-called telophasic split (SHARP 1929, ROBERTSON 1931, and KAUFMANN 1925) did not exist here; and that the spongio-reticular structures in the nucleus, commonly figured in sections from mass fixations, were artifacts of slow fixation. BELAR (1929a, 1929b)

came to a similar conclusion. The writer found (like MARTENS 1927, 1929, and BELAR 1929a) that living resting (non-dividing) nuclei (in cells showing cyclosis), which would not normally divide again, when examined in an aqueous medium by the best water-immersion objectives, with accurate microscopy, were seen to contain unsplit, loosely coiled and zigzagged chromonemas. The plant material in which this was observed included: stamen-hairs of *Tradescantia virginiana*, of *Tradescantia fluminensis* (in which the nuclei sometimes appeared blank, but at other times showed the chromonemas), and of *Rhoeo discolor;* the hairs on the labellum of *Cypripedium pubescens*, and the stinging hairs of *Urtica gracilis*. When such non-dividing nuclei were fixed and stained, with optimum fixation, the same unsplit chromonemas were seen in them; but with slow fixation the familiar spongio-reticular structure (seen in sections of material fixed in mass) regularly appeared.

Preparations made by sectioning from material fixed in bulk suffer from too slow fixation. In the telophases, chromatin is being lost, and it is not strange that the interior of the vanishing chromosome should sometimes be more or less unstained, and that by poor fixation a moniliform aspect should be caused. It seems to the writer premature to infer from such changes that there is a longitudinal split in the telophase. For no signs of such a split are seen in good smear preparations of telophases, as observed by BELAR (1929b) and by the writer.

In the leptotene of Lilium, Galtonia, Allium, Scilla, Hyacinthus, Tulipa, and Agapanthus, in smear preparations stained with iron-brazilin and showing the chromomeres, it was ascertained by the writer that there was no trace of a longitudinal or other split, in either the chromomeres or their connecting fibers. It was also seen clearly that the secondary split came subsequently, in mid pachytene; early pachytene having only the primary split (figure 1). In Allium, slow destaining (in hyrax) of the early pachytene (1931a) proved that each chromomere had only one stained submicroscopic core, which was, in the writer's opinion, a gene with a thin covering of chromatin. There being only one such core in a chromomere, in the early pachytene, proved, apparently conclusively, that the secondary split had not yet appeared.

Hence, the evidence from the best fixed preparations, namely smear preparations showing chromomeres, is against any general telophasic split; and the supposed split can be sometimes explained as an artifact of slow fixation, resulting from fixation in mass.

Crossing over probably occurs during pachytene

In Lilium the average longitudinal distance between the centers of adjacent chromomeres at late pachytene (figure 2) was under half a micron. It

was seen to be less than this in several other liliaceous plants (but not in Fritillaria). Hence a relative movement of the homologous chromonemas by half a micron would bring about unequal crossing over, if any crossing over should occur. But unequal crossing over seems rare, being only known in the bar locus. Thus, apparently, crossing over must take place at pachy-

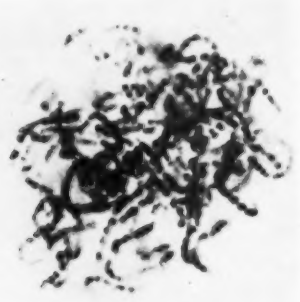

FIGURE 1.—Early pachytene of *Lilium regale*. Smear preparation, fixed in Navashin's mixture, stained with brazilin, and mounted in immersion oil. Pressed nearly flat. Photographed with Zeiss apochromatic 90 of 1.4 aperture, with homal. Enlargement near 1300. The chromomeres have *nearly* all synapsed. The secondary split has not yet appeared.

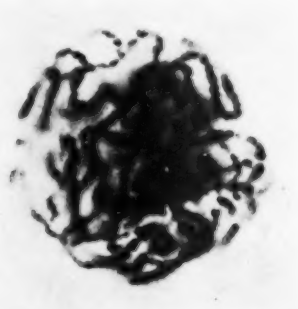

FIGURE 2.—Late pachytene of *Lilium regale*. Preparation as in figure 1, but not pressed. Focus somewhat below upper surface. All chromomeres have previously synapsed, and all are bilobed by the secondary split.

tene, when the homologous chromonemas are *fixed* together in the right positions by the connections between homologous chromomeres across the primary split. Any opening-out of the primary and secondary splits, or any sliding of the chiasmas, would apparently prevent that exact correspondence of the homologous chromomeres in the two homologous chromonemas, necessary to obviate unequal crossing over.

The two chromioles formed by the secondary split are equivalent

When a chromomere with its contained gene divides into two, genetic work has shown that there is no difference between the two products. One is apparently a replica of the other, as far as the evidence goes. Hence neither of them can truly be called the "old" chromomere or chromiole, or the "new" chromomere or chromiole. The same applies to chromonemas, which are strings of chromomeres. Two adjacent chromomeres at a certain minute distance apart, which is usually less than half a micron from center to center, can mutually form a connecting fiber. Such a fiber appears at zygotene between approaching homologous chromomeres, at first (sometimes) in the form of minute projections from the two adjacent chromomeres. Only one such fiber unites a pair of homologous chromomeres or a pair of chromioles. When the chromomeres divide at the secondary split, at mid pachytene, then a new fiber passes between two of the homologous chromioles transversely to the primary split. The new and the old fiber may thus form two transverse fibers at each locus. When the secondary split appears, a new *longitudinal* fiber is also formed. This passes between two adjacent non-homologous chromioles. The old fiber connects the other two adjacent chromioles. In all cases these fibers pass *the shortest way*, between chromioles that are nearly or quite touching; and do not pass diagonally, because this would be about four-tenths longer. (Thus there would not be normally half twists formed between sister chromonemas.)

Opening-out at earliest diplotene is (sometimes) at the primary split, and at first at many points

At earliest diplotene (schizotene) in *Allium triquetrum*, the homologous chromonemas of the nine bivalents begin to separate at about two hundred points (1931a), a number which is in excess of the total chiasmas, which may amount to near twenty at late diaphase. A large number of points of opening-out has also been seen at early diplotene (schizotene) in Lilium. At this stage, some of the pachytene chromonemas will not yet have opened-out completely, and all details of the process of opening-out can be traced (figure 3). In no case has the secondary split been seen to open-out in Lilium. (The chiasmas become visible at pachytene, before any opening-out.) The primary split is broad and clear, while the secondary split is hard to see (and has been so from its origin at mid pachytene), only consisting in the two-lobed condition of the chromomeres. In no case in liliaceous plants did four separate "opened-out" threads appear, as it would seem (as already stated) might occur at the points between the alternate openings-out presumed to exist in certain grasshoppers. McCLINTOCK (1931) proved that, in deficiencies in maize, only the primary split

showed at late pachytene at the point where the deficiency was. The same was the case with inversions. If the secondary split also opens out at diplotene, it is in bivalents which, at diaphase and metaphase, show normally no chiasmas but only terminal junctions (Datura).

In the supposed "break and join" of two or more chromonemas, the "break" apparently does not occur

In crossing over, translocation, interchange, inversion, and deletion, it has been supposed that the chromonemas first broke and then joined up in a different way. Since the breaks must correspond, in crossing over (as already stated), with an accuracy of less than half a micron, they are unlikely. Since breaks are presumably due to a tension, such tension will

FIGURE 3.—Stage (*L. pardalinum*) between pachytene and diplotene (early diplotene or "schizotene"). Prepared as in figure 1, but photographed with objective of 1.3 aperture. Slightly flattened. Focus just below surface. Thick (double) and thin (single) threads. In Lilium the opening-out seems to be only at the primary split.

render immediately subsequent joins improbable. Hence it is likely that the only disconnections that occur in chromonemas are between half of the newly formed chromioles, before they form a longitudinal connection with their nearest neighbors. In other words, chromonemas may join up from an unjoined state, but do not normally break after having joined.

ORIGIN OF CHIASMAS

Division of chromomeres, and formation of new fibers

For some time after the chromomeres have divided, at mid pachytene, only half the final number of longitudinal connecting fibers are clearly visible in each of the two synapsed chromonemas (as the writer has seen especially in Lilium). They are the old fibers. When the new fibers appear between the remaining half of the divided chromomeres (chromioles),

they are at first thin and inconspicuous. But they become indistinguishable from the old fibers at late diplotene (figure 4). Thus when the chromomeres of the two synapsed threads divide each into two chromioles, as they do in mid pachytene, there are only sufficient longitudinal threads for half of them. Each old chromomere forms two new chromioles, and there are no old chromomeres, old chromioles, old chromonemas, or old chromosomes left; only old connecting fibers are visible. Now, these old fibers cannot pass obliquely (as already stated) without an increase in length of about 40 percent. They do not seem to increase in length, but remain directly longitudinal between two of the chromioles (*which* two chromioles is apparently determined by chance). When, after a short time and during late pachytene, the *new* longitudinal fibers start their growth, they are forced

FIGURE 4.—Stage (*L. pardalinum*) when the opening-out is just complete (late diplotene, or "diplotene"). Preparation as in figure 1. Viewed below the upper surface. The bivalents form nodes and internodes. A node is not necessarily a chiasma, though many are.

to pass directly longitudinally because of the direct longitudinal position of the old fibers. Hence, there are, normally, no oblique connecting fibers between sister strands (in meiosis or mitosis); and no crossing over, twisting or overlapping of the sister strands.

Overlaps of the two homologous chromonemas

Overlaps of the two synapsing homologues seem likely to take place often enough to form the usual numbers of chiasmas. They will only form the diagonal X's if the chromomeres are sufficiently apart; for the formation of an X reduces the longitudinal distance between the centers of the chromomeres by about 30 percent. Thus if the centers of the chromomeres are 0.5 micron apart, and the connecting fibers are 0.2 micron long; then an overlap would form an X, with the chromomeres 0.05 micron apart. But if the chromomeres at 0.5 micron distance were connected by fibers only

0.1 micron long, then they could not form the diagonals of an X because these old connecting fibers would not be long enough. In this case the half twist or overlap would perhaps remain more or less external, without causing an X.

The X of the overlap may arise at zygotene with either of two directions of turn. When the chromomeres divide into two, the two diagonals of the X, like the normal direct longitudinal connections between the other chromomeres of the synapsed chromonemas, cannot become oblique, because this would require a growth of about 40 percent over the previous length. Hence they each remain in a plane with one of the two pairs of non-sister chromonemas (halves of synapsed partners). Which of the two planes any diagonal of the X is in, is apparently settled by chance. Thus the two diagonals may remain: (1) in both directions of overlap, together

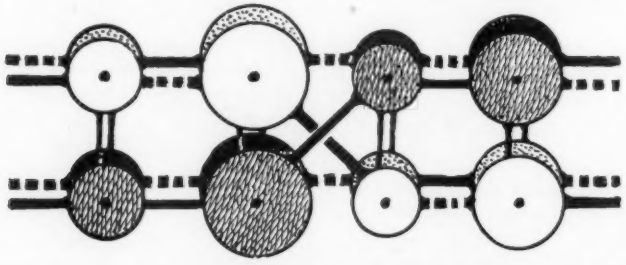

FIGURE 5.—Diagram of a direct chiasma, as seen at pachytene in Lilium. The solid lines are the old connecting fibers, the broken lines the new ones.

above; (2) in both directions of overlap, together below; (3) with one direction of overlap, *a* above and *b* below; or (4) with the other direction of overlap, *b* above and *a* below. These four kinds of X will occur equally frequently by chance, only if overlaps in different directions are equal in number. The first two kinds may be classed together as forming *direct* chiasmas (and direct crossovers), and the second two kinds as forming *oblique* chiasmas (and oblique crossovers). The writer has identified both kinds of chiasmas at pachytene in Lilium, in about equal numbers.

DIRECT AND OBLIQUE CHIASMAS

Direct chiasmas

Direct chiasmas may occur by chance in half of the overlaps which form chiasmas (this is shown by the study of ANDERSON'S genetic results with attached X's, which give equal numbers of direct and oblique chiasmas). When the two diagonals of an X are in the same plane at pachytene (as the writer has observed them sometimes in Lilium), then the two pairs of chromioles in the other plane are fairly close together longitudinally

(figure 5), and the new fibers are formed between these chromioles. (We may call the two sister chromonemas of one homologue, a and a'; and those of the partner b and b'.) This will give two direct crossover strands, $a+b$ and $b+a$ (or the converse), as well as two non-crossover strands, forming the diagonals of the X, namely a' and b' (or the converse). If we adopt the convention that the chromonemas a and b are above, and a' and b' below, we have: with the X above, direct crossovers $a'+b'$ and $b'+a'$, and non-crossovers a and b; and with the X below, direct crossovers $a+b$ and $b+a$, and non-crossovers a' and b'.

Oblique chiasmas

Here one diagonal of the X is above and one below, as the writer has sometimes observed in Lilium (figure 6), thus forcing the new longitudinal

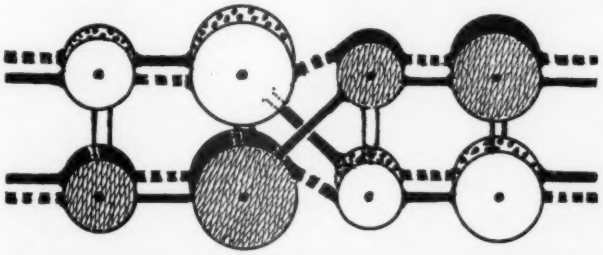

FIGURE 6.—Diagram of an oblique chiasma, as seen at pachytene of Lilium.

fibers to pass obliquely on each side between the unattached chromioles. (The old fibers of course cannot pass obliquely, because to do so they would have to increase 40 percent in length.) Here also the diagonals do not cross over, and the oblique chromonemas do. There are then two cases (given by the two directions of overlap), in which the top diagonal slants either from upper left to lower right, or from lower left to upper right. Diagonals a and b' give oblique crossovers $a'+b$ and $b+a'$, with non-crossovers a and b'. With the diagonals b and a', we have the oblique crossovers $a+b'$ and $b'+a$, with the non-crossovers b and a'.

Adjacent chiasmas

With two adjacent chiasmas we may have eight cases (and also eight converse cases, made by changing a to a', b to b', a' to a, and b' to b).

CHIASMAS	CROSSOVERS	
(1) Direct (A)+supplemental direct (A).	$\frac{a+b}{b+a}+\frac{a+b}{b+a}$	2 similar supplemental chiasmas.
(2) Direct (A)+complemental direct (B).	$\frac{a+b}{b+a}+\frac{a'+b'}{b'+a'}$	2 similar complemental chiasmas.

(3) Oblique (C)+supplemental oblique (C).	$\frac{a+b'}{b'+a}+\frac{a+b'}{b'+a}$	2 similar supplemental chiasmas.
(4) Oblique (C)+complemental oblique (D).	$\frac{a+b'}{b'+a}+\frac{a'+b}{b+a'}$	2 similar complemental chiasmas.
(5) Direct (A)+oblique (C).	$\frac{a+b}{b+a}+\frac{a+b'}{b'+a}$	2 unlike chiasmas.
(6) Direct (A)+oblique (D).	$\frac{a+b}{b+a}+\frac{a'+b}{b+a'}$	2 unlike chiasmas.
(7) Direct (B)+oblique (C).	$\frac{a'+b'}{b'+a'}+\frac{a+b'}{b'+a}$	2 unlike chiasmas.
(8) Direct (B)+oblique (D).	$\frac{a'+b'}{b'+a'}+\frac{a'+b}{b+a'}$	2 unlike chiasmas.

The supplemental similar chiasmas produce each two double crossovers and two non-crossovers. The complemental similar chiasmas give four single crossovers each. The four unlike pairs of chiasmas give each one double crossover, two single crossovers, and one non-crossover chromatid.

The four similar pairs of chiasmas have been called compensating, because the jugate chromatids, after crossing over, regularly separate or come together alternately, so that the two (conjunct) threads on one side of an internode adjacent to the left of the first chiasma are continuous with the two threads on one side of an internode adjacent to the right of the second chiasma. So too, the four unlike pairs are sometimes called non-compensating, because the jugate chromatids do not alternate in arrangement, and so two threads on the left of the first chiasma which are on the same side (conjunct) after diplotene will not appear to be conjunct on the right of the second chiasma.

Sometimes the double crossovers in cases of pairs of similar supplemental chiasmas (A+A, and C+C) are called "reciprocal". The double crossovers from the four pairs of unlike chiasmas (A+C, A+D, B+C, and B+D) have been called "diagonal"; but only one chiasma and crossover in each seems diagonal, and C+C is perhaps the truly diagonal chiasma pair.

Chiasmas and crossovers

Direct and oblique single chiasmas can be shown to be about equally numerous. With regard to pairs of chiasmas, when an overlap in one direction takes place, the next overlap, it seems probable, would occur in the opposite direction; for if it was in the same direction it would cause a twist, which seems unlikely, especially when the ends of the chromonemas synapse first. Hence usually there should be alternation of overlaps, and if every overlap forms a chiasma, there would be an alternation of direction of the overlaps of the diagonals of the X's in sequent chiasmas. This would not affect chance crossing over in direct chiasmas; but with a pair of se-

quent oblique chiasmas the results for crossing over would apparently differ from chance ones. For two sequent oblique chiasmas would most often be complemental rather than supplemental. This would lessen the number of double crossovers expected by chance, for complemental pairs of chiasmas have no double crossovers. If, however, we temporarily disregard this possible source of error, we have by chance 8 pairs of chiasmas occurring with equal frequencies. Having regard to the direction of overlap, we get 32 different pairs of chiasmas; but for the following we may consider only 8 main classes. These give 32 chromatids, and it can be reckoned (table 1) that there are normally, by chance, 8 non-crossovers, 16 single crossovers, and 8 double crossovers, among these.

TABLE 1

CHIASMAS	CHROMOSOMES		
	NON-CROSSOVERS	SINGLE CROSSOVERS	DOUBLE CROSSOVERS
Direct+supplemental direct	2	..	2
Direct+complemental direct	..	4	..
Oblique+supplemental oblique	2	..	2
Oblique+complemental oblique	..	4	..
2 (Direct+oblique)	2	4	2
2 (Oblique+direct)	2	4	2
Totals	8	16	8
Proportion	1	2	1

Thus the proportion for two sequent chiasmas, by chance, is 1 non-crossover, to 2 single crossovers, to 1 double crossover. With a third adjacent chiasma, the proportion would be (1:2:1) (1:1); or 1 non-crossover, to 3 single crossovers, to 3 double crossovers, to 1 triple crossover. For four sequent chiasmas, the chance proportion would be (1:2:1) (1:2:1); or 1 non-crossover, to 4 single crossovers, to 6 double crossovers, to 4 triple crossovers, to 1 quadruple crossover; and so on. (The percentage figures of the crossover chart of a chromosome are made to include the double crossovers, though these do not normally affect the recombinations.) So we have the results given in table 2, for bivalents with terminal fusal chromomeres, or single arms of J or V chromosomes. The distal recombinations are the sums of the odd-crossover chromosomes.

However, it does not seem commonly to happen that the same number of chiasmas occurs in all homologous bivalents. Thus the total chart crossovers may be somewhere between the numbers given in table 2. For the X chromosome of Drosophila, the numbers of crossovers in 100 chromosomes have long been published (see MORGAN 1926).

Chromosomes	Percentages	
Non-crossovers	43.5	Total chart crossing over = 70.5
Single crossovers	43	Distal recombinations = 43.5
Double crossovers	13	
Triple crossover	0.5	

Comparing this with the writer's chiasma theory, we note that the distal recombinations are 6.5 below 50 percent. Also the non-crossovers are in excess of the single crossovers, while the opposite happens on the chiasma theory (table 2). Taking the figures as given, we may deduce the following:

	Percentages
No-chiasma bivalents	13
One-chiasma bivalents	37
Two-chiasma bivalents	46
Three-chiasma bivalents	4

TABLE 2

Chart figures and recombinations in bivalents with terminal fusal attachment.

NUMBERS OF CHIASMAS	PERCENTAGES OF CROSSOVERS					CHART CROSSOVERS	DISTAL RECOMBINATIONS
	0	1	2	3	4		
(0)	(100)	..	..	..	..	(0)	(0)
1	50	50	..	..	..	50	50
2	25	50	25	..	..	100	50
3	12.5	37.5	37.5	12.5	..	150	50
4	6.25	25	37.5	25	6.25	200	50
And so on.							

However, since there is a proximal third or more of the X chromosome without sufficient mutants to detect double crossing over, it is possible that double crossing over is slightly more abundant than found. Perhaps some few distal crossovers are also undetected. No-chiasma bivalents do not seem to occur in attached X's, where their presence could be ascertained (see table 4). Hence, to agree with the writer's chiasma theory (neglecting the rare triple crossovers), the percentage of single crossovers should be increased by 7, and that of double crossovers by 2.

ATTACHED AND RING X CHROMOSOMES

Attached X's and crossing over

In the female Drosophila, three of the chromatids of the X bivalent usually pass into the polar bodies. So we cannot normally get both of the crossover chromatids from any crossover. But in the attached X's we regularly get two (non-sisters) of the four chromatids, and these serve to

show the distinction between direct and oblique crossing over. Direct crossovers, in attached X's, are between the two chromosomes of the V; and oblique crossovers are between two V's (but not between sister chromatids). If the attached X's are originally heterozygous for several loci, then single direct chiasmas do not change this heterozygosis: but single oblique chiasmas (that is 1/2 of the single chiasmas) make all heterozygous loci distal to them homozygous in the resulting attached X's. That is, if all chasmas are single, 50 percent of the attached X's (if heterozygous originally) are homozygous distal to the chiasmas. But, with all chiasmas single, 50 percent of the resulting chromosomes show one crossover, and 50 percent are non-crossovers. Hence the percentage of single-crossover chromosomes from single chiasmas is equal to the percentage of distally homozygous attached X's from single chiasmas. The percentage of *recessive* distally homozygous attached X's is half this (or half the percentage of single crossovers from single chiasmas).

Of the eight kinds of double chiasmas (table 1), two, both proximal direct plus distal oblique, give distal homozygosis of originally heterozygous attached X's. Of the 16 resulting attached X's, two are homozygous for recessives; that is, 12.5 percent. These 16 attached X's have 32 chromosomes, of which 8 (on the average) are non-crossovers, 16 are single crossovers, and 8 are double crossovers. This would yield a crossover chart total of 100 percent crossing over. It gives 25 percent double crossovers. Thus the percentage (due to double chiasmas) of attached X's distally homozygous for recessives is half the double crossovers (or a quarter of the total chart single crossovers due to double chiasmas).

Now the crossovers in the X of *Drosophila melanogaster* may be taken as (excluding the rare triple crossing over, and also the possibility of no-chiasma bivalents) nearly equal to,

Non-crossovers, 35 percent	Total chart crossing over = 80.
Single crossovers, 50 percent	
Double crossovers, 15 percent	Distal recombinations = 50.

These would arise from:

Single chiasmas, 40 percent
Double chiasmas, 60 percent

The distal recessive homozygosis for 100 resulting attached X's would be half the crossovers from single chiasmas, 20/2 = 10 percent, plus half the double crossovers, 15/2 or 7.5; totaling 17.5 percent. The proximal parts of the attached X's, with about 10 percent of chart crossovers, should give about 10/2, or 5 percent of recessive homozygosis; since there are few or no proximal double crossovers to be reckoned with here.

Since the total chart crossovers are made up of the total single crossovers (s) plus twice the double (d) crossovers (that is, $s+2d=C$), the recessive distal homozygosis is $\frac{s-2d}{2}+\frac{d}{2}=\frac{s-d}{2}=\frac{50-15}{2}=17.5$; or $=\frac{C-3d}{2}=\frac{80-45}{2}=17.5$. STURTEVANT'S value for the *sc* locus is 17.1. The higher value of 19 for the locus *y* is probably due to differential viability (STURTEVANT 1931).

The values for *f* and *g* in table 3 (calculated from single chiasmas only) are over the values found. (It seems possible that there were a few double chiasmas proximal to these.) The data do not permit of the calculation of the values from *m* to *ec*.

TABLE 3

MUTANT LOCI	PROXIMAL CROSSOVERS FOUND	PERCENTAGES OF DISTALLY HOMOZYGOUS RECESSIVE ATTACHED X'S FOUND	PERCENTAGES CALCULATED FROM PROXIMAL CROSSOVERS AND CHIASMAS	
f	13.5	5.1	6.8	Mainly single crossovers
g	26	10.3	13.0	
m	33.9	13.5		Single and double crossovers
v	37	14.8		
t	42.4	16.1		
ct	50	16.4		
cv	56.3	15.9		More double crossovers
ec	64.5	16.5		
sc	70	17.1	17.5	
y	70	(19)	17.5	

For ANDERSON'S important experiments (1925), the attached X's may be divided into 3 regions: (A) from the fusal (spindle) chromomere to the locus of *f*, chart distance = 13.5; (B) from *f* to *ct*, chart distance = 36.5; and (C) from *ct* to *sc* (presumably near the distal end), chart distance = 20. In A the double crossovers are apparently unknown; in B they are ascertained; and in C they have not been measured in this experiment. However, we have assumed, for the X chromosome, that the total double crossovers are 15 percent. Those with both or the second crossovers in B are 5 percent. Thus 10 percent have their second crossovers in C. (Hence we can remove the second of the double crossovers from the chart distance in B and C, and consider only the proximal crossovers.)

Of the 13.5 single crossovers in A, 6.75 are probably oblique and produce homozygosis; and 6.75 would be direct. Taking the total double crossovers in B and C as 15, we may subtract the second (distal one) of these in

sections B and C; B having 5 known, and C therefore having the remaining 10.

TABLE 4

SECTION	CROSSOVERS IN ATTACHED X'S	ATTACHED CHROMOSOMES AND CHIASMAS
A	13.5	Direct, complementary non-crossovers, 6.75 Oblique, identical non-crossovers, 6.75
B	36.5−5=31.5	Direct, 15.75 { complementary crossovers, 7.88 complementary non-crossovers, 7.88 Oblique, 15.75 { crossovers and non-crossovers non-crossovers and crossovers } 15.75
C	20−10=10	{ Direct, Oblique, } complementary non-crossovers, 10

Thus we have the totals of attached X's:

(1) Complementary non-crossovers = 6.75 + 7.88 + 10 = 24.63
(2) Identical non-crossovers = 6.75
(3) Crossover and non-crossover = 15.75
(4) Complementary crossovers = 7.88

From this we get table 5. The fit would be somewhat closer if one or

TABLE 5

	CALCULATED FROM CHART DISTANCES	ANDERSON'S RESULTS FROM ATTACHED X'S
(1) Complementary non-crossovers	44.8	43.9
(2) Identical non-crossovers	12.3	10.8
(3) Crossover and non-crossovers	28.6	29.7
(4) Complementary crossovers	14.3	15.6

more of the double crossovers was found to be included in the 13.5 chart distance from *f* to the proximal end; for the chief difference is in the 5.4 percent of oblique crossovers found proximal to *f*, giving 10.8 (oblique + direct) crossovers; whereas the crossover chart has 13.5 crossovers here.

Ring X's and crossing over

L. V. MORGAN (1932, and *in litt.*) showed that when a ring X synapses with another ring X (or with a rod X) double-crossover X chromosomes survive, but no single crossovers appear in the progeny. Two chromonemas crossing over once would form one large ring (or one long rod) with two fusal chromomeres. This does not survive in the progeny. Double crossovers would form two rings (or a ring and a rod) and survive. But two

sister chromonemas crossing over would also form one inviable large ring (or long rod). If sister-strand crossing over occurred, the numbers of non-crossover chromosomes would be diminished with regard to the numbers of double-crossover chromosomes. This is not the case. Therefore sister-strand crossing over does not occur (normally) between two ring X's (or between the ring and the rod X). Hence it was not postulated above to solve the problem of attached X's, nor would it fit these. Therefore it probably does not occur in normal X's.

EXPLANATION OF GENE REARRANGEMENT

Reversed crossing over

This appears to originate usually at meiosis, and in a normal trisomic plant (Datura); but less commonly in a diploid (1927). On the writer's theory, if the fusal chromomeres synapsed, but the rest of the two chromonemas was in reverse order, crossing over could take place only close to the fusal chromomeres. Such reversed crossing over would give two chromosomes; one with two left arms, and the other with two right arms. The third homologous chromosome, in the trisomic plant, might help this process by synapsing first with one or both ends of one of the chromonemas concerned. Reversed crossing over in Datura appears to have occurred only next the fusal chromomere.

Reciprocal translocation

If any two heterologous chromonemas overlapped close enough when their chromomeres were first dividing, there might be (by the writer's theory) cross-connections found between two of the four, as in an ordinary chiasma (but not between sisters). Such cross-connections would produce reciprocal translocations, in which the interchanged parts were neither equal nor homologous. A bend at the point of overlap might result in a gene or more being omitted at the point of interchange.

It has been suggested that heterologous interchange results from interlocking of bivalents at meiosis. This seems possible; but it is also possible that some cases styled "interlocking" at meiosis may be due to interchange. If interchange occurs at *mitosis*, it cannot apparently be due to interlocking.

Terminal translocation

The writer has seen, in meiosis, the ends of the chromonemas resting laterally on other chromonemas. If cross-connections like those at a chiasma occurred during longitudinal divisions, chromonemas would result with terminal attachment of a heterologous segment. This might also occur at mitosis.

Inversion and deletion

If a dividing mitotic chromonema (or a bivalent at early pachytene) overlapped itself closely in a loop, when its chromomeres divided there might be formed cross-connections between two threads, as in a chiasma. This would result in an inversion (or a deletion). The deleted piece may form a ring (McCLINTOCK 1933). It is readily seen how a gene might be lost in the inversion, by the connecting fiber passing it by.

Insertion

This is perhaps a double interchange, where a loop of one chromonema overlaps another chromonema at two near places. The piece lost may be small. Insertion should be quite rare, since it requires two rare interchanges to cause it.

Terminal deficiency

This seems common. There are several possible ways in which it may arise. Every terminal translocation, for instance, is accompanied by a terminal deficiency in another chromonema.

OBJECTIONS TO THE THEORY

The following objections have lately been made to the present writer's modified Janssens' theory of crossing over; which accounts also for translocation, deficiency, interchange, inversion, deletion, and insertion.

First Objection. That direct chiasmas formed at overlaps, according to the writer's theory, can at most result in 50 percent of chromatids showing crossing over; whereas some chromosomes of Drosophila actually show a greater percentage than this. *Reply.* If the two pairs of sister chromatids at crossing over are a and a', and b and b', respectively; and a is opposite to b, and a' is opposite to b'; then, at the overlaps, direct chiasmas may be formed in which a crosses over with b, and also by chance, in equal numbers direct chiasmas in which a' crosses over with b'. So the four chromatids from a bivalent which has more than one direct chiasma, may sometimes all be crossovers; and it is possible that, in some cases of long chromosomes, the limit of 100 percent of crossover chromosomes may be reached. (Oblique chiasmas are left out of consideration here; but would, of course, add to the numbers of crossover chromosomes.)

Second Objection. That, on the writer's theory, the pairs of sister chromatids formed by the secondary split at meiosis should show half as many apparent twists as there are genes in the chromosome; and the same should happen between the two halves of a split chromosome in any mitotic division. *Reply.* It is a fact of observation that the longitudinal connection fibers between chromioles pass the shortest way (GELEI 1921, BELLING 1931a). The shortest way between any two homologous chromioles of a split chromonema and their two neighbors on either side is not obliquely

but longitudinally. Hence the old fiber will be longitudinal, and so force the new one to be so. Thus, also, there will be (normally) no twists between sister strands, though there may be rarely twists of the whole chromosome or bivalent. The difficulty with the objector, and this holds also for the first objection, is that there is no *old* chromiole when a chromomere has divided. The *old* chromomere has gone, and there are two new chromioles formed, which are equivalent. So also the old chromonema when divided changes into two new chromonemas. (There are, however, old connecting fibers, and subsequently new ones also, between adjacent chromioles.)

Third Objection. That there is always or usually a telophasic split in somatic divisions, and also that the leptotene of meiosis is always or usually split. *Reply.* A telophasic split has been predicated mainly by observers who have used sections of plant (or animal) material, fixed *en masse.* This method, in the writer's opinion, does not show fine nuclear details truly, but gives the alveolar artifacts of slow fixation. This is proved by comparison with similar material made into smear preparations by the best methods (see BELAR 1929a, 1929b). The writer has spent some months with smear preparations of mitosis (both diploid and haploid), especially of Tradescantia and Rhoeo, and has convinced himself that there is no telophasic split visible in his material. He is also satisfied that there is no longitudinal split to be seen, with good microscopy, in the leptotene threads of the liliaceous plants he has examined.

Fourth Objection. That bivalents with unequal homologues are inconsistent with the writer's theory of crossing over. *Reply.* It was the behavior of such bivalents which first led the writer to discredit the alternate-opening-out hypothesis of the origin of chiasmas. The effects of opening-out in these bivalents are visible at early diaphase. They do not show, in the cases examined, opening-out of the sister chromatids at the unequal free ends. They should show this is about half the cases, on the alternate-opening-out hypothesis, when sliding of the X's of chiasmas had not taken place. In WENRICH's excellent figures of Phrynotettix (1916), all the bivalents with unequal homologues show at early diaphase that the unequal extremities have not opened out at the secondary split. The writer has observed this same fact in a bivalent of Aloë with unequal homologues.

At the first anaphase, with a terminal (or sub-terminal) fusal point as in Phrynotettix, if there is no crossing over, or if there are two similar supplemental chiasmas, then the two long chromatids separate from the two short chromatids and pass to opposite poles (as in some of WENRICH's figures). But if there is one chiasma, a long and a short chromatid pass together to each pole (as in other figures of WENRICH). The explanation of this is that there has been crossing over. The alternate-opening-out hypothesis of chiasma origin apparently cannot apply here, as already shown.

Fifth Objection. That the reduction in the number of nodes observed in some plants, from early diplotene to metaphase, is due to the breaking and rejoining of chiasmas; not to the disappearance of some loose twists, or overlaps, or of temporary lateral fusions. *Reply.* Since the nodes (apparently mostly temporary chromatin cross-connections) at early diplotene in *Allium triquetrum* seem somewhat over 200 in number (1931a), while the chiasmas at diaphase may be about 20, a loss of about 180 nodes results. It does not seem probable that these many lost nodes were chiasmas. For nodes are stated not to be lost in Stenobothrus from late diplotene to late diaphase (DARLINGTON and DARK 1932). Nor should it be assumed without proof that a node is a chiasma. That chiasmas break and join is improbable, for the writer knows no case of chromosomes joining after proved fracture. On the writer's hypothesis there is normally no fracture in crossing over, translocation, interchange, inversion, deletion, deficiency, or insertion.

Sixth Objection. If non-disjunction of the X chromosomes in Drosophila is attributed to the X's having so many chiasmas that they fail to disjoin; then, in the high non-disjunction line, why do the X's show less crossing over instead of more? *Reply.* The first part of this objection is a conjecture. Since the high non-disjunction is admittedly due to translocation or inversion, it seems probable that the apparent non-disjunction is sometimes non-conjunction, as the writer has noted in triploids and tetraploids (and also in Uvularia). If there are cases of whole or partial non-conjunction or asynapsis (compare MCCLINTOCK 1931), then the percentage of crossing over would be decreased.

Seventh Objection. The opening-out of the X of a chiasma towards the ends of the bivalent must separate the two chromosomes at this end, instead of giving a terminal junction. *Reply.* Since the terminal chromomeres visibly hold the leptotene-zygotene chromonemas together at the distal ends in a number of organisms, it is not improbable that the same distal chromomeres may hold the four jugate chromatids together when brought into contact at the ends. In this case the opening-out of the X of a chiasma at diplotene when it reaches the end of a bivalent will bring the terminal chromomeres of the chromatids into close proximity, and connection by new cross threads may result.

Eighth Objection. That the percentage of homozygosis of recessive alleles that were heterozygous in the parent, towards the distal (left) end of the attached X's of Drosophila, was found by ANDERSON, and also by others, to be slightly more than that calculated for random assortment of the recessive alleles. This was said to be inexplicable on the writer's hypothesis. *Reply.* The writer's hypothesis gives different grades of homozygosity of recessive alleles, according to the amount crossing over from proxi-

mal single chiasmas, equalling half of these crossovers, since only oblique (not direct) chiasmas cause homozygosis of heterozygous alleles in attached X's. There must be added to this half the double crossovers. For the X chromosome, terminal recessive homozygosity is about 17.5 percent on the writer's theory.

Ninth Objection. In the translocation of a terminal piece of one arm of the third chromosome to the fourth chromosome of Drosophila, crossing over was less in the proximal part of the attached portion of the third chromosome, in flies homosomic for the translocation, than in the normal flies. This was said to be inexplicable on the writer's theory. *Reply.* If synapsis begins with the small fourth chromosome in the combined piece (as it should, since the fusal point of the third chromosome is left behind), then there will be interference between this point and the first chiasma, and few crossovers will occur near this end; as perhaps happens also near the proximal end of the X, and near the fusal points of the second and third chromosomes (see also DOBZHANSKY 1931).

Tenth Objection. In haploid Zea, a split was found in the threads of the meiotic prophases. It was objected that this was contrary to the hypothesis of the writer, which is based on observations of an unsplit leptotene. *Reply.* The leptotene stage is more difficult to demonstrate than the pachytene, especially in aceto-carmine smears. The pachytene stage of the diploid Zea has been figured and photographed by MCCLINTOCK; but good figures and photographs of the leptotene are yet to be sought. The writer would suggest that further work might perhaps show a stage in the haploid Zea comparable with the leptotene of the diploid, as the split stage found in the haploid is perhaps comparable with the late pachytene of the diploid Zea.

Eleventh Objection. That a tertiary split has been demonstrated in the pachytene of one plant, and also that there were two split spiroid chromonemas in the first anaphase chromosomes. That this is contrary to the writer's hypothesis, in which the pachytene has only two splits, and the first anaphase chromosomes show two unsplit chromonemas. *Reply.* When the late pachytene is well enough fixed to show the chromomeres distinctly, only two splits are visible in Lilium and Fritillaria. Preparations which are to demonstrate a hitherto invisible tertiary split should perhaps show the chromomeres as distinctly as those obtained by the writer.

The spiroid threads found in the metaphase and anaphase chromosomes by appropriate destaining may perhaps appear with lighter centers when their chromatin is disappearing, as is the case from anaphase to telophase. Most workers however have found that the first-anaphase spiroid threads are only two in number. That this is the case in Rhoeo and Tradescantia, the writer also can testify, from iron-brazilin and iron-haematoxylin smears, fixed with Navashin's or with Flemming's mixture.

Twelfth Objection. That the genetic proof (PATTERSON 1933) of the chromosomes in the sperms of Drosophila being probably sometimes split, furthers the hypothesis of a telophasic split, and so is against the writer's hypothesis. *Reply.* The dense accumulation of chromatin in the sperms of most animals and some plants usually hides the state of the chromonemas. But when the sperm nucleus spreads out in the fertilized ovum, in plants and animals, it is usually found to be in the later prophase, and at a stage when the chromosomes are normally split. Hence the splitting of chromonemas may possibly take place in the spermatozoön. In a certain nematode, according to MULSOW, the chromosomes in the sperm are at the metaphase stage, and doubtless already split. PATTERSON's results also show that the chromonemas in the sperm are sometimes (in six-sevenths of the cases) *not* split, which is contrary to the "telophase-split" hypothesis of some writers.

Thirteenth Objection. That the chromomeres found in the pachytene of diploid Zea (by the acetic-alcohol and aceto-carmine method) do not correspond in the two synapsed threads. *Reply.* These "chromomeres" are often compound chromomeres run together during fixation, and then may be composed in different ways in the two threads. The same thing happens in Lilium, Fritillaria and Aloë, if the fixation is not rapid enough.

Fourteenth Objection. That no crossing over between sister chromatids was found either between two ring X's or between a normal and a ring X, in Drosophila. *Reply.* Sister-strand crossing over was, because of this fact, excluded from the writer's theory, which now uses equal numbers of direct and oblique chiasmas only, as calculated from ANDERSON's results with attached X's, and confirmed by observation of the pachytene of Lilium.

Fifteenth Objection. That the writer's theory does not fully explain ANDERSON's results with attached X's and equational exceptions. *Reply.* In the first form of the writer's hypothesis these could be only approximately explained by diagonal and sister-strand assortment at the spindle-fiber end. The present form of the theory enables ANDERSON's numerical results to be calculated with fair accuracy (see above).

Sixteenth Objection. That the late pachytene of Zea shows more nodes to a chromosome than the chiasmas seen at diaphase. *Reply.* A chiasma presumably arises only at an overlap, which is a kind of half-twist with no rotation in each chromonema. If the chromomeres are too close together, an overlap or half-twist may not result in a chiasma. Also at earliest diplotene, besides chiasmas, there are many (often hundreds of) chromatin connections across the primary split. A few of these may remain and hold the threads together for a time.

Seventeenth Objection. Since the action of X-rays of definite frequency in causing point mutation is taken as indicating that the bare genes are too

small to be visible with the microscope, therefore the genes cannot be directly counted. *Reply*. The writer's observations in destaining ultimate chromomeres in hyrax have shown that each contained one minute stainable central body (like a centriole). The cells were examined with the monobromide of napthalin objective, at a working aperture of 1.5. The central particles were probably over 0.07 micron across. They were doubtless covered with chromatin. The bare gene is then smaller than this. Hence, also, with regard to mutation, since the gene substance in one chromomere is not divisible by crossing over, this must be regarded as a whole; that is, as one gene. Whether the whole, or only part, of a gene is acted on by the X-rays does not seem generally ascertained. But in the formation of multiple alleles the action must apparently be partial. In this case the size of the whole gene does not seem deducible from the (direct or indirect) action of X-rays on a part of it.

Eighteenth Objection. It has been estimated that *Drosophila melanogaster* has over six times as many genes as the writer found in Lilium, and as seem to be about the number of ultimate chromomeres indicated in the grasshopper Phrynotettix (WENRICH 1916). *Reply*. This estimation seems to the writer to mingle point mutations with gene rearragements (deficiencies, inversions, interchanges) as causing dominant or recessive lethals. It also does not allow for the sperm chromonemas having sometimes split especially in the autosomes. Hence the resulting figure is too high (see PATTERSON 1933, MCCLINTOCK 1931).

SUMMARY

1. The writer's modification of JANSSENS' hypothesis explains crossing over, and also explains gene rearrangements, such as reversed crossing over, reciprocal translocation, inversion, deletion, and deficiency.

2. The chromonemas were proved to be unsplit at leptotene in certain plants.

3. Living (and also fixed) chromonemas of resting "final" nuclei showed no split, in the plants examined.

4. The secondary split was first seen at mid pachytene.

5. Both direct and oblique chiasmas were seen at pachytene in Lilium.

6. After the chromomeres have split, the old longitudinal fibers are either alone visible; or are seen to be thicker than the new ones.

7. In Lilium the opening-out at diplotene seems to be only at the primary split. (However, in plants such as Datura, with no chiasmas at diaphase, it is probable that the diplotene opening-out alternates at the chiasmas.)

8. Since chiasmas arise at pachytene in certain liliaceous plants, they cannot arise from alternate opening-out at diplotene.

9. Chiasmas seem to be due to overlaps, not twists. Overlaps may be sometimes mistaken for twists, under the microscope.

10. There are 8 main kinds of double chiasmas, equally numerous by chance.

11. Double chiasmas give, by chance, one non-crossover chromosome, two single-crossover chromosomes, and one double-crossover chromosome.

12. If crossovers arise from chiasmas, then the distal recombinations from the end to the fusal chromomere should be 50 percent.

13. If crossovers arise from chiasmas, then the chart crossovers divided by 50 should give the average number of chiasmas.

14. The ascertained numbers of crossover X chromosomes of *Drosophila melanogaster* appear to lack about 7 percent of single crossovers, and about 2 percent of double crossovers, if they arose from chiasmas (neglecting triple crossovers).

15. Flies with heterozygous attached X's in Drosophila (ANDERSON 1925), should (on the writer's theory) give distal recessive homozygotes in a percentage equal to half the chart length minus one and a half times the percentage of double crossovers. This would be 17.5.

16. On the writer's theory, the percentages of complementary and identical non-crossovers, of crossovers plus non-crossovers, and of complementary crossovers, in attached X's, have been calculated from the chromosome chart, and agree with ANDERSON's experimental results.

17. L. V. MORGAN's work with ring X's in Drosophila, like ANDERSON's results with attached X's, shows the absence of sister-strand crossing over. Therefore chiasmas formed by a half twist between the two homologues are absent, or rare (1931b).

18. It is possible to explain reversed crossing over, heterologous interchange, terminal translocation, inversion, deletion, and deficiency, by the overlapping of two chromonemas when their chromomeres are dividing. The result is equivalent to the formation of a chiasma between synapsed homologues; but is less regular, so that genes may be lost at the junctions.

19. Some objections to the writer's theory are briefly answered.

LITERATURE CITED

ANDERSON, E. G., 1925 Crossing over in a case of attached X chromosomes in *Drosophila melanogaster*. Genetics **10**: 403–417.

BATESON, W. and PUNNETT, R. C., 1911 On gametic series involving reduplication of certain terms. J. Genet. **1**: 293–302.

BELAR, K., 1929a . . . Untersuchungen an den Spermatocyten von Chorthippus . . . W. R. Arch. EntwMech. **118**: 359–484.

1929b Untersuchungen an den Staubfadenhaarzellen und Blattmeristemzellen von *Tradescantia virginica*. Z. Zellforsch. **10**: 73–134.

BELLING, J., 1927 The attachments of chromosomes at the reduction division in flowering plants. J. Genet. **18**: 177–205.

1928 The ultimate chromomeres of Lilium and Aloe . . . Univ. California Publ Bot. **14:** 307–318.

1931a Chromomeres of liliaceous plants. Univ. California Publ. Bot. **16:** 153–170.

1931b Chiasmas in flowering plants. Univ. California Publ. Bot. **16:** 311–338.

BELLING, J. and BLAKESLEE, A. F. 1924 The configurations and sizes of the chromosomes in the trivalents of 25-chromosome Daturas. Proc. Nat. Acad. Sci. Washington **10:** 116–120.

CREIGHTON, H. B. and MCCLINTOCK, B., 1931 A correlation of cytological and genetical crossing over in *Zea mays*. Proc. Nat. Acad. Sci. Washington **17:** 485–497.

DARLINGTON, C. D. and DARK, S. O. S., 1932 The origin and behavior of chiasmata. II. *Stenobothrus parallelus*. Cytologia **3:** 169–185.

DOBZHANSKY, T., 1930 Translocations involving the third and the fourth chromosomes of *Drosophila melanogaster*. Genetics **15:** 347–399.

1931a The decrease of crossing over observed in translocations and its probable explanation. Amer. Nat. **65:** 214–232.

1931b Translocations involving the second and the fourth chromosomes of *Drosophila melanogaster*. Genetics **16:** 629–658.

DOBZHANSKY, T. and STURTEVANT, A. H., 1931 Translocations between the second and third chromosomes of Drosophila and their bearing on Oenothera problems. Contrib. to the genetics . . . of Drosophila . . . Publ. Carnegie Instn. Wash. **421:** 29–59.

EMERSON, S. and BEADLE, G. W., 1932 Experiments with attached-X chromosomes. Proc. Sixth Int. Congress Genetics **2:** 48–49.

GELEI, J., 1921 Weitere Studien über die Oogenese des *Dendrocoelum lacteum* . . . Arch. Zellforsch. **16:** 88–169.

JANSSENS, F. A., 1909 La théorie de la chiasmatypie. Nouvelle interpretation des cinèses de maturation. Cellule **22:** 387–411.

KAUFMANN, B. P., 1925 The existence of double spiral chromatin bands and a "bouquet" stage in *Tradescantia pilosa* . . . Amer. Nat. **59:** 190.

KUWADA, Y., 1932 The double coiled spiral structure of chromosomes. [Japanese with English summary.] Bot. Mag. Tokyo **46:** 307–310.

MCCLINTOCK, B., 1930 A cytological demonstration of the location of an interchange between two non-homologous chromosomes of *Zea mays*. Proc. Nat. Acad. Sci. Washington **16:** 791–796.

1931 Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Missouri Agric. Expt. Sta. Res. Bull. **163:** 1–30.

1932 A correlation of ring-shaped chromosomes with variegation in *Zea mays*. Proc. Nat. Acad. Sci. Washington **18:** 671–681.

MARTENS, P., 1927 Recherches expérimentales sur la cinèse dans la cellule vivante. Cellule **38:** 69–174.

1929 Nouvelles recherches expérimentales sur la cinèse dans la cellule vivante. Cellule **39:** 169–215.

MENDEL, G., 1866 Versuche über Pflanzenhybriden. Verh. naturf. Ver. Brünn. **4.**

MORGAN, L. V. 1932 Genetic behavior of a closed X chromosome of Drosophila. Proc. Sixth Int. Congress of Genetics **2:** 135–137.

MORGAN, T. H., 1911 An attempt to analyze the constitution of the chromosomes . . . J. Exp. Zool. **11:** 365–413.

1926 The theory of the gene. Yale Univ. Press.

MULLER, H. J., 1916 The mechanism of crossing over. Amer. Nat. **50:** 193–221, 284–305, 350–366, and 421–434.

MULLER, H. J. and PAINTER, T. S., 1932 The differentiation of the sex chromosome of Drosphila into genetically active and inert regions. Z.I.A.V. **62:** 316–365.

OFFERMANN, C. A. and MULLER, H. J., 1932 Regional differences in crossing over as a function of the chromosome structure. Proc. Sixth. Int. Congress Genetics **2:** 143–145.

PAINTER, T. S. and MULLER, H. J., 1929 Parallel cytology and genetics of induced translocations and deletions in Drosophila. J. Hered. **20:** 287–298.

PATTERSON, J. T., 1929 The production of mutations in somatic cells of *Drosophila melanogaster* by means of X-rays. J. Exp. Zool. **53:** 327–372.

1933 The mechanism of mosaic formation in Drosophila. Genetics **18:** 32–52.

RHOADES, M. M., 1931 The frequencies of homozygosis of factors in attached-X females of *Drosophila melanogaster*. Genetics **16:** 375–385.

ROBERTSON, W. R. B., 1931 . . . Synapsis in the Tettigidae . . . J. Morph. **51:** 119–145.

SHARP, L. W., 1929 Structure of large somatic chromosomes. Bot. Gaz. **88:** 349–382.

SAX, K., 1932 The cytological mechanism of crossing over. J. Arnold Arboretum **13:** 180–212.

STERN, C., 1931 Zytologisch-genetische Untersuchungen als Beweise für die Morgansche Theorie des Faktorenaustauschs. Biol. Zbl. **51:** 547–587.

STURTEVANT, A. H., 1915 The behavior of the chromosomes as studied through linkage. Z.I.A.V. **13:** 234–287.

1931a Known and probable inverted sections of the autosomes of *Drosophila melanogaster*. Contrib. to the genetics . . . of Drosophila . . . Publ. Carnegie Instn. Washington **421:** 1–27.

1931b Two new attached-X lines of *Drosophila melanogaster*, and further data on the behavior of heterozygous attached-X's. Contrib. to the genetics . . . of Drosophila. . . . Publ. Carnegie Instn. Washington **421:** 61–81.

STURTEVANT, A. H. and DOBZHANSKY, T., 1930 Reciprocal translocations in Drosophila, and their bearing on Oenothera cytology and genetics. Proc. Nat. Acad. Sci. Washington **16:** 533–536.

SUTTON, W. S., 1903 The chromosomes in heredity. Biol. Bull. **4:** 231–251.

WENRICH, D. H., 1916 The spermatogenesis of Phrynotettix . . . Bull. Mus. Comp. Zool. (Harvard) **60:** 57–135.

1917 Synapsis and the chromosome organization in Chorthippus . . . and Trimerotropis . . . J. Morph. **29:** 471–516.